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MINOR ALKALOIDS OF *LYCOPODIUM ANNOTINUM L.*

by

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ABSTRACT

The minor alkaloids of Lycopodium annotinum L. have been investigated by elution chromatography.

The spectroscopic and chemical evidence which lead to the determination of the constitution and stereochemistry of the dinitrogeneous Lycopodium alkaloids, α-obscurine, β-obscurine, and lycodine is presented.

The constitution and stereochemistry of the Lycopodium alkaloid L-8, which we named lycodoline, has been determined.

The characterization of a previously unreported alkaloid of Lycopodium annotinum L. which we have named annopodine is described.

A biosynthetic scheme for the Lycopodium alkaloids utilizing lysine and isopentenyl pyrophosphate is presented.

CONTENTS

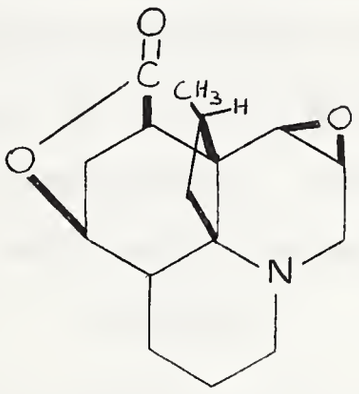
I N T R O D U C T I O N

The presence of alkaloids in the Lycopodiaceae was first demonstrated in 1881 by Bodeker (1) who isolated an alkaloid, which he called lycopodine, from Lycopodium complanatum L. Only sporadic attention was paid to the alkaloids of these plants throughout the following sixty years. In 1934, Orechoff (2) detected the relatively high concentration of alkaloids in Lycopodium annotinum Linn. and in the following year, Muszynski (3) reported that alkaloids were present in three additional species. Achmatowicz and Uzieblo (4), isolated three crystalline alkaloids, including lycopodine, from Lycopodium clavatum Linn.

Manske and Marion, in the period 1942 - 1948, carried out an extensive survey involving the isolation and characterization of approximately 35 alkaloids from eleven distinct species. This work demonstrated that the occurence of alkaloids in the Lycopodiaceae is quite general and provided a firm basis for subsequent researches concerned with further isolations and structural determinations of these alkaloids. Manske has provided a review (5) which tabulates the results of the above survey as well as the results of other workers of the period (6,7).

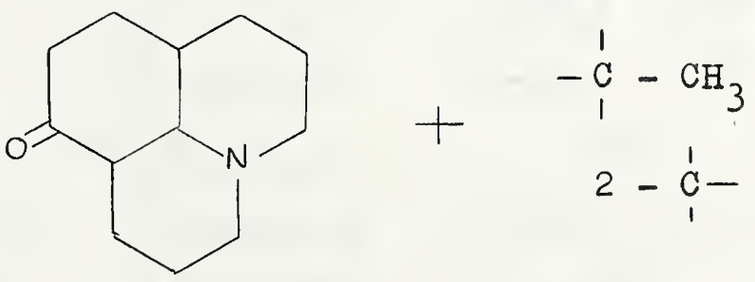
The first structural elucidation of a Lycopodium alkaloid was achieved by Wiesner and co-workers (8,9) in the period 1953 - 1957 with annotinine, the major alkaloid of Lycopodium annotinum Linn. The relative

stereochemistry followed in 1958 on the basis of chemical arguments (10) and x-ray analyses (11,12). The combined chemical and physical evidence indicated structure I to be the correct representation of the alkaloid.



I (Annotinine)

Lycopodine, the most widespread alkaloid of the series, was the next to receive comprehensive experimental attention. A proposal for the partial structure II was made by MacLean and Harrison (13) in 1959.



II

In 1958, work was initiated in these laboratories on

the Lycopodium alkaloids, in particular on the structure of lycopodine (14). In 1959, with the lycopodine problem well underway, we turned our attention to some of the minor alkaloids of this family.

In particular, we were attracted by the rich alkaloidal content of Lycopodium annotinum Linn. Up to and including 1959, several groups of workers had reported on the isolation of alkaloids from the species. The alkaloids isolated and the corresponding references are shown in Table I.

TABLE I

ALKALOID	MOLECULAR FORMULA	MELTING POINT	REFERENCE
Nicotine	$C_{10}H_{14}N$	Dipicrate 226°	16
Unnamed	$C_{10}H_{19-21}ON$	B-CH_3I 290°	6
Annotine (L-11)	$C_{16}H_{21}O_3N$	176°	18
Annotinine (L-7)	$C_{16}H_{21}O_3N$	232°	18
Lycodine	$C_{16}H_{22}N_2$	119°	20
Base VIII	$C_{16}H_{21}O_3N$	B-CH_3I 217°	16
Base IV	$C_{16}H_{23}ON$	B-CH_3I 265°	16
Acrifoline (L-27)	$C_{16}H_{23}O_2N$	103°	6
Base VI (L-29)	$C_{16}H_{23}O_2N$	B-CH_3I 294°	16
Lycopodine	$C_{16}H_{25}ON$	116°	18
Isolycopodine	$C_{16}H_{25}ON$	136°	17
Unnamed	$C_{16}H_{25}ON$	B-$HClO_4$ 234°	6
Lycofoline	$C_{16}H_{25}O_2N$	145°	19
Annofoline	$C_{16}H_{25}O_2N$	157°	19

TABLE I (Continued)

ALKALOID	MOLECULAR FORMULA	MELTING POINT	REFERENCE
Lycodoline (L-8,L-30)	$C_{16}H_{25}O_2N$	180°	18
L-9	$C_{16}H_{25}O_2N$	122°	18
L-10	$C_{16}H_{27}ON$	B-$HClO_4$ 223°	18
β-Obscurine	$C_{17}H_{24}ON_2$	318°	18
Base IX	$C_{17}H_{25}O_2N$	B-CH_3I 324°	16
Base X	$C_{17}H_{25}O_3N$	B-CH_3I 315°	16
α-Obscurine	$C_{17}H_{26}ON_2$	284°	18
Base V (L-28)	$C_{17}H_{27}O_2N$	B-CH_3I 304°	16
Base XI	$C_{18}H_{25}O_3N$	B-CH_3I 272°	16
Base XII	$C_{18}H_{25}O_4N$	B-CH_3I 283°	16
O-Acetyl Acrifoline (L-12)	$C_{18}H_{25}O_3N$	120°	18
Tawcettiine	$C_{18}H_{29}O_3N$	B-CH_3I 297°	19
Lofoline	$C_{18}H_{29}O_3N$	212°	19
Base VII (L-31)	$C_{20}H_{29}O_4N$	B-CH_3I 292°	16
Annotoxine (L-11 + L-27)	$C_{32}H_{44}O_5N_2$	197°	6

Examination of Table I shows that while most of the reported alkaloids contain a single nitrogen atom, this pattern is departed from in three cases (α-obscurine, $C_{17}H_{26}ON_2$; β-obscurine, $C_{17}H_{24}ON_2$; lycodine, $C_{16}H_{22}N_2$). It appeared possible that the dinitrogeneous alkaloids

might be related and that their structures might constitute some degree of departure from the known annotinine skeleton and the emerging lycopodine skeleton.

The experimental evidence and the necessary interpretation which allows the complete elucidation of the structures of these alkaloids is presented in detail in the following discussion.

The second part of the discussion is concerned with the evidence for the structure of lycodoline (L-8, L-30), one of the monoacidic bases listed in Table I.

D I S C U S S I O N

I. ISOLATION OF THE ALKALOIDS

The crude alkaloidal material of Lycopodium annotinum Linn was obtained by the method reported by Manske and Marion (21). A methanol extract of the dried, ground plant material was reduced in volume by distillation until a viscous oil remained. The basic and water soluble material was separated from water insoluble material by prolonged stirring with dilute hydrochloric acid. The aqueous acid solution was separated from water insoluble material by filtration.

The filtrate thus obtained was washed thoroughly with ether to remove neutral contaminants, made basic (pH 10-11) with ammonium hydroxide, and thoroughly extracted with chloroform. The chloroform solution was evaporated to small volume and the remaining chloroform was replaced with 95% ethanol.

The volume of the ethanol solution was then reduced until crystallization began. Filtration yielded annotinine (25% of the crude alkaloid of this species). Evaporation of the mother liquors left a viscous golden brown oil, subsequently referred to as "annotinine free" crude alkaloid, which was the starting point for our investigations.

Bertho and Stoll (6), Achmatowicz and Rodewald (16,17), and Manske and Marion (18), used various combinations of fractional crystallization of free bases, fractional

crystallization of various salts, solvent extraction, and distillation to isolate the alkaloids listed in Table I. Anet and co-workers (19, 20), used countercurrent distribution in conjunction with elution chromatography to effect the separation of the several alkaloids in Table I which had remained undetected by the more traditional methods of the earlier workers.

Our preliminary separations, utilizing elution chromatography and infrared and ultraviolet spectoscopy to monitor the fractions, followed by fractional crystallization of both the free bases and their salts from suitably combined fractions, showed that the alkaloids in which we were interested could be isolated from the remaining material with reasonable ease.

After the general chromatographic behaviour of a particular alkaloid was determined, its relative concentration was increased by applying a quantity of "annotinine free" crude alkaloid to a column of alumina such that the ratio of alumina to alkaloid mixture was of the order of 10-15 to 1. The chromatogram was then developed by elution with a series of solvents of increasing polarity. For example, the column would be eluted with benzene until the eluate contained essentially no alkaloid. This would be repeated with ether, ethyl acetate, chloroform and chloroform-methanol (9:1). The fraction containing the desired alkaloid (as determined by spectroscopic measurements) would then be subjected to more careful chromatography (larger ratio of

adsorbent to compound, more cautious development). The fractions produced in this way were treated individually or combined, according to their similarity as shown by their infrared and/or ultraviolet spectra, and were then treated with various solvents to effect crystallization of the free bases. When the free bases ceased to crystallize, advantage was taken of salt formation to obtain crystalline material. When the salts ceased to crystallize, the mother liquors were treated to recover the free bases and, if necessary, chromatographed again. It was also often necessary to chromatograph the crystalline material or the bases recovered from crystalline salts because of the tendency of certain of the alkaloids to cocrystallize.

While we were primarily interested in the isolation of α- and β-obscurine, lycodine and lycodoline, many other alkaloids were isolated at the same time. Among these were annotine, annotinine, acrifoline, lycopodine, o-acetylacrifoline, fawcettiine, lofoline and annotoxine (complex of annotine and acrifoline). The alkaloids of Table I which remained undetected were nicotine, the two unnamed alkaloids of Bertho and Stoll ($C_{10}H_{19-21}ON$ and $C_{16}H_{25}ON$), isolycopodine, lycofoline, annofoline, L-9, L-10 or any of the alkaloids designated by Roman numerals. The latter were isolated by Achmatowicz and Rodewald (17) who treated the mother liquors of Lycopodium annotinum Linn with methyl iodide and then fractionally crystallized the resulting salts to obtain the various bases in the form

of their methiodides. We made no attempt to repeat this procedure. Our chromatographic method has, however, provided two previously unreported alkaloids. The first, which we have named annopodine, is a C_{17} methyl ester ($C_{17}H_{25}O_3N$, M.P. 212°) whose characteristics will be described later in this discussion. The second, which we have temporarily designated alkaloid 2-A, is another methyl ester ($C_{17}H_{25}O_3N$, M.P. 123°) which has also been isolated at the University of New Brunswick (22). Its structure is at present the subject of a co-operative research effort by the U.N.B. group and this laboratory.

2. THE STRUCTURES OF α AND β-OBSCURINE.

α-And β-obscurine are the components of a molecular complex called obscurine which has been isolated from L. annotinum L. (18). L. Flabelliforme (21), and L. obscurum Var. dendroideum (24). The complex (M.P. 282°) cannot be separated into its components by fractional crystallization but Moore and Marion (25) showed that the components, α-obscurine (M.P. 282°, $C_{17}H_{26}ON_2$), and β-obscurine (M.P. 317°, $C_{17}H_{24}ON_2$), could be separated by elution chromatography over alumina. We have obtained the individual components by the chromatographic procedure outlined at the beginning of this discussion. The alkaloidal material obtained from a chromatogram of the "annotinine free" crude alkaloid by elution with ethyl acetate and chloroform was rechromatographed over alumina. α-Obscurine

was detected in the fractions eluted by ethyl acetate, ethyl acetate-chloroform and chloroform by means of its characteristic ultraviolet absorption spectrum (λ max = 255 mμ) (25). The alkaloid was isolated from appropriately combined fractions by crystallization from acetone. β-Obscurine was usually obtained from the last fraction (eluted with chloroform:methanol 9:1) of the initial chromatogram by trituration with acetone. α-Obscurine was further purified by recrystallization from acetone while β-obscurine was purified by recrystallization from methanol or methanol-chloroform solution.

Initial structural studies on α- and β-obscurine were carried out by Moore and Marion (25). In addition to defining the molecular formulae ($C_{17}H_{26}ON_2$ and $C_{17}H_{24}ON_2$ respectively) they showed that α-obscurine contained one secondary nitrogen (infrared absorption at 3411 cm^{-1}) and an amide carbonyl (infrared absorption at 1667 cm^{-1}). They further suggested, on the basis of the ultraviolet absorption spectrum (λ max 255 log ϵ = 3.75), that the amide carbonyl is also a part of an α,β-unsaturated system, although no adequate model was available in the literature at that time to support this. They also provided the very valuable observation that the dehydrogenation of α-obscurine by palladium-charcoal gives 7-methylquinoline and 6-methyl-2-pyridone. The identities of the two degradation fragments were established by comparison with authentic materials.

β-Obscurine was shown to contain a secondary nitrogen and a 2-pyridone system by comparison of the infrared and ultraviolet absorption spectra of β-obscurine and 6-methyl-2-pyridone. The pertinent data is tabulated below:

INFRARED ($CHCl_3$)

COMPOUND	Frequency (cm^{-1})
β-Obscurine	3385, 1659, 1651, 1620, 1613
6-Methyl-2-pyridone	3387, 1662, 1651, 1628, 1613

ULTRAVIOLET

COMPOUND	λ max	Log ϵ
β-Obscurine	232, 315	3.98, 3.89
6-Methyl-2-pyridone	229, 304	3.87, 3.83

The nature of the other nitrogen atom was not discussed but their analysis indicated the absence of an $\geq N-CH_3$ group in both α- and β-obscurine.

The mode of occurence, closely related molecular formulae, and the degradation of α-obscurine to 6-methyl-2-pyridone prompted Moore and Marion to attempt the conversion of α-obscurine to β-obscurine by bromination and

dehydrobromination. They reported that the experiment was a failure but supplied no experimental details.

Anet and Eves (20) in a preliminary study of the structure of lycodine, the third dinitrogeneous base occurring in Lycopodium annotinum Linn, suggested that a close relationship might exist between lycodine and β-obscurine. In an effort to support this, they attempted, without success, to acetylate β-obscurine (a N-acetyl derivative of lycodine had previously been obtained). The experiment however suggested that the basic nitrogen in β-obscurine is tertiary.

The first step in our investigation of these alkaloids was a thorough analysis of the purified compounds. These analyses confirmed the molecular formulae as defined by Moore and Marion but indicated the presence of one $\geq N\text{-}CH_3$ group in each alkaloid. α-Obscurine gave somewhat less than one mole of volatile acid on Kuhn-Roth oxidation indicating the presence of a single $\geq C\text{-}CH_3$ group in the alkaloid.

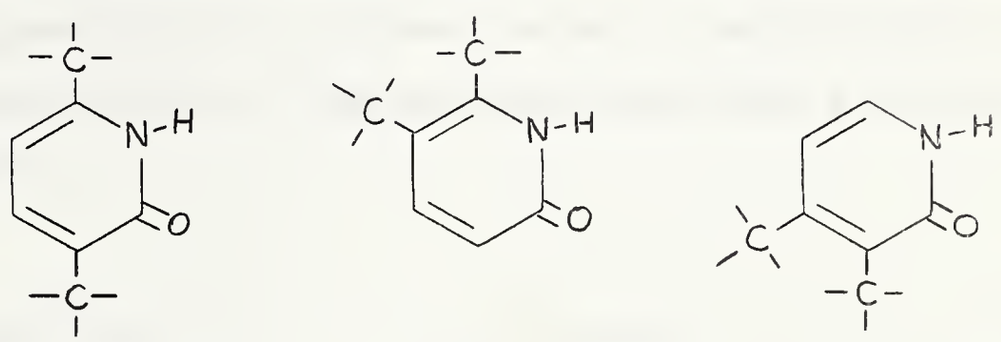
The nuclear magnetic resonance (N.M.R.) spectrum of α-obscurine shows a 3-proton signal at $\tau = 7.55$ ($\geq N\text{-}CH_3$) (26) and a 3-proton signal at $\tau = 9.14$ (doublet $J = 5$ c.p.s.) indicating the system $\geq CHCH_3$ (26). The N.M.R. spectrum of β-obscurine shows a 3-proton signal at $\tau = 7.46$ ($\geq N\text{-}CH_3$) and a 3-proton signal at $\tau = 9.17$ (broadened singlet) indicating the $\geq C\text{-}CH_3$ group.

The N.M.R. spectra thus provide confirmation for the

presence of an N-methyl group in each alkaloid, indicate that the volatile acid produced by Kuhn-Roth oxidation of α-obscurine stems from a secondary C-methyl group, and indicates the presence of a C-methyl group in β-obscurine.

The N.M.R. spectrum of β-obscurine also shows signals at low field appearing as a pair of doublets centered at τ = 2.21 and τ = 3.63 reminiscent of a typical AB quartet (J_{AB} = 10 c.p.s.).

Since the infrared spectrum of β-obscurine shows >N - H stretching absorption (25), and the basic nitrogen is tertiary (20), the pyridone nitrogen must then be unsubstituted. The combined spectral data indicated that β-obscurine must be a 3,4-disubstituted, a 3,6-disubstituted, or a 5,6-disubstituted 2-pyridone. The choices appear in Figure IV.



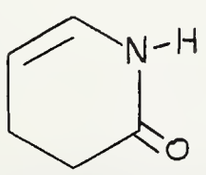
3,6-disubstituted 5,6-disubstituted 3,4-disubstituted

IV

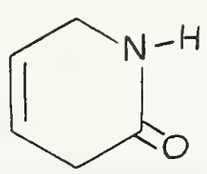
Attempts to acetylate α-obscurine with acetic anhydride-pyridine met with failure, suggesting that again the

basic nitrogen is tertiary. Since the infrared spectrum of α-obscurine shows >N-H stretching absorption, the amide nitrogen must be secondary. Along with the carbonyl absorption at 1675 cm^{-1}, there is a medium band at 1700 cm^{-1} which may be attributed to a >C = C< stretching vibration. Since the N.M.R. spectrum of α-obscurine shows at low field only one broad signal (τ = 1.99) which may be attributed to the hydrogen on the amide nitrogen (26), it follows that the double bond which is conjugated with the amide system (ultraviolet absorption λ max 255, log ϵ = 3.73) is tetrasubstituted.

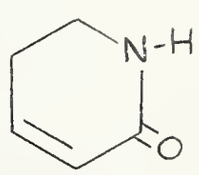
Since α-obscurine gives 6-methyl-2-pyridone on dehydrogenation (25) and contains two more hydrogen atoms than β-obscurine it appeared, as Moore and Marion had suggested (25), that the chromophoric system of α-obscurine is merely a reduced 2-pyridone. If this is true, then three possibilities (without regard to substitution) exist for that portion of the structure and these are shown in Figure V.



3,4-dihydro-
2-pyridone



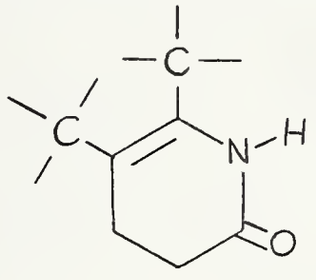
3,6-dihydro-
2-pyridone



5,6-dihydro-
2-pyridone

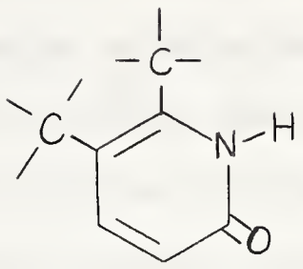
V

The 3,6-dihydro-2-pyridone system may be eliminated because it should not absorb strongly in the ultraviolet above 220 mμ, since the unsaturated portions are not conjugated. Edwards and Singh (27) have reported the ultraviolet absorption spectrum of 6-methyl-5,6-dihydro-2-pyridone (λ max 235 mμ, log ϵ = 3.15; λ max 241 mμ, log ϵ = 3.17) which appears to eliminate the 5,6-dihydro system. Thus by elimination, the 3,4-dihydro-2-pyridone system appeared to be the most attractive possibility. In view of the tetrasubstituted nature of the carbon-carbon double bond (N.M.R.) and of the production of 6-methyl-2-pyridone on dehydrogenation, the reduced 2-pyridone system of α-obscurine could be extended to VI.



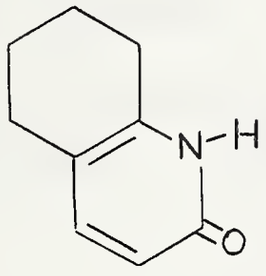
VI

If VI is correct for the chromophoric system of α-obscurine and if the assumption that α-obscurine is the reduced 2-pyridone analogue of β-obscurine, is also correct, then the 2-pyridone system of β-obscurine must be substituted as in Figure VII.

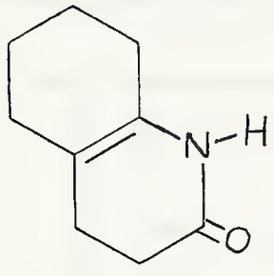


VII

The evidence which shows that these interpretations and assumptions are correct was provided by the interrelation of the two alkaloids by chemical methods and spectroscopic comparison with two model compounds; 5,6,7,8-tetrahydro carbostyril(VIII)and 3,4,5,6,7,8-hexahydrocarbostyril(IX)(28).



VIII



IX

α-Obscurine was converted in 30% yield into β-obscurine by treatment with N-bromosuccinimide in refluxing carbontetrachloride.

N-Bromosuccinimide has found widespread application in carrying out brominations at allylic positions and

positions α to carbonyl groups (29). In the case of α-obscurine, the dehydrobromination takes place either during the reaction or during the workup in which the system is exposed to aqueous sodium hydroxide. The β-obscurine isolated from the reaction product was identical (M.P., M.M.P., infrared and ultraviolet spectra) to authentic material.

The transformation of β-obscurine into α-obscurine was accomplished by the reduction of β-obscurine with lithium in liquid ammonia. This reagent has been shown by Berson and Walia (30) to selectively reduce 2-pyridones to the corresponding 3,4-dihydro-2-pyridones.

The model compounds used for spectroscopic comparison were prepared (28) by known methods; in the case of 5,6,7,8-tetrahydrocarbostyril, the procedure of Dornaw and Neuse (31) was followed. The spectral data obtained are shown below.

SPECTRUM	β-Obscurine Absorption Bands	5,6,7,8-Tetrahydro carbostyril Absorption Bands
Infrared (Nujol)	3320,1667,1635, 1560 cm^{-1}	3270,1660,1630, 1554 cm^{-1}
Ultraviolet (Ethanol)	232 mμ ($\log\epsilon$ = 3.98) 315 mμ ($\log\epsilon$ = 3.89)	230 m ($\log\epsilon$ = 3.88) 315 m ($\log\epsilon$ = 3.89)
N.M.R. ($CDCl_3$)	τ = 2.21,3.63(centres of doublets) J = 10 c.p.s.	τ = 2.79,3.67(centres of doublets) J = 9 c.p.s.

Elvidge and Jackman (23) have published the chemical shift data for the 2-pyridone system and their work is in agreement with the assignment of the absorption at τ = 3.63 to the proton at the 3-position of the 2-pyridone ring. The hydrogen at the 4-position of the 2-pyridone in β-obscurine is deshielded, presumably by some portion of the molecule which is not present in the model compound.

The model compound, 3,4,5,6,7,8-hexahydrocarbostyril, was prepared by the method of Campbell and Stevens (32); the spectral comparison with α-obscurine is tabulated below.

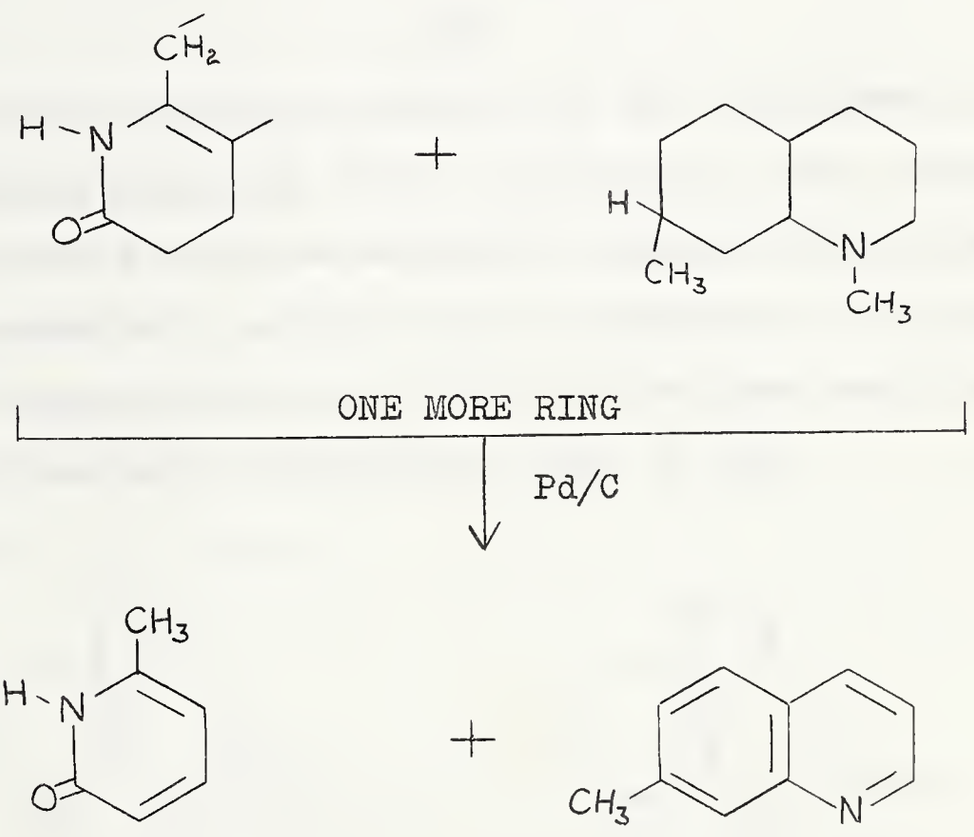
SPECTRUM	α-Obscurine Absorption Bands	3,4,5,6,7,8-Hexahydrocarbostyril Absorption Bands
Infrared (Nujol)	3280, 3200, 3120, 1700, 1667 cm^{-1}	3300, 3200, 3100, 1705, 1667 cm^{-1}
Ultraviolet (Ethanol)	255 mμ ($\log \epsilon$ = 3.73)	253 mμ ($\log \epsilon$ = 3.67)
N.M.R. ($CDCl_3$)	τ = 1.99 (one proton-broad)	τ = 1.94 (one proton-broad)

The interconversion described above and the comparison of the alkaloids with the model compounds proves the relationship which exists between them and shows that apart from the chromophoric system, the remainder of the structure is common to both compounds.

The interconversion also clarifies the discrepancy which is apparent in the N.M.R. spectrum of β-obscurine. The 3-proton signal at τ = 9.17 in the spectrum of β-obscurine is in the form of a somewhat broadened singlet. This, at first sight, would indicate a tertiary C-methyl group (26), however the N.M.R. spectrum of α-obscurine (τ = 9.14; J = 5 c.p.s.; 3 protons) indicates that α-obscurine possesses the $>CHCH_3$ system. It is apparent then, that the broadening of the singlet in the spectrum of β-obscurine is significant, and must indicate an unresolved doublet due to a secondary C-methyl group in the molecule. This will be discussed in greater detail later.

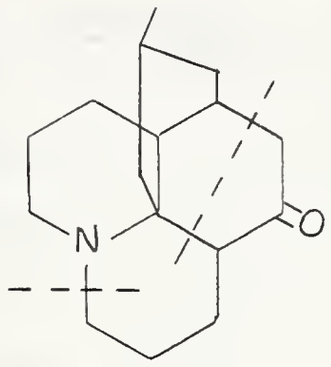
The fragments (7-methylquinoline and 6-methyl-2-pyridone) obtained from α-obscurine on dehydrogenation become more significant in the light of the information presented above. The methyl group at the seven position of the quinoline and the methyl group at the six position of the 2-pyridone cannot arise from the same carbon atom (assuming no deep seated rearrangements during the reaction) since the methyl groups occupy different positions in relation to the nitrogen atoms and the original methyl group in α-obscurine is not located on a double bond (N.M.R. spectrum). If α-obscurine contained a methyl group at the six position of the 3,4-dihydro-2-pyridone system, the N.M.R. spectrum should show a sharp 3-proton signal in the region 8.00-8.50 (26). Since it was shown above that α-obscurine contains a 3,4-dihydro-2-pyridone system substituted at the 5 and 6 positions, and that the substituent at the

6 position is not a methyl group, then the 6 methyl group in the dehydrogenation pyridone must be formed during the dehydrogenation. If the assumption is made that the methyl group attached to the basic nitrogen is eliminated in the formation of the 7-methylquinoline, then all the carbon, nitrogen, and oxygen atoms which make up the formula are accounted for, and if the further assumption is made that the methyl group in the 6-methyl-2-pyridone arises from a methylene group in the original molecule, then the problem reduces to one of uniting the two fragments shown below in such a way as to form one more ring.



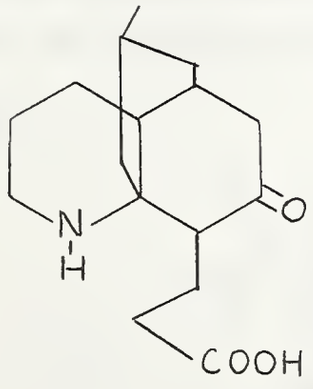
Some insight into a possible way in which these fragments could be joined together was suggested by the complete structure of lycopodine (XII) which became known (15) while

the structural work on α- and β-obscurine was underway. Lycopodine also gives 7-methylquinoline on dehydrogenation (33), probably by the cleavage indicated by the dashed line in XII.

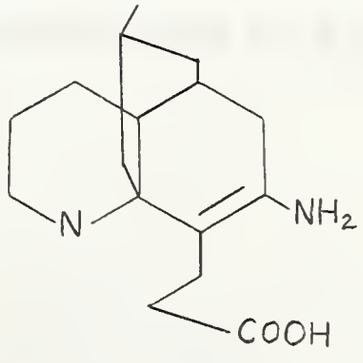


XII

Since lycopodine occurs with the obscurines in Lycopodium annotinum Linn, a relatively close biogenetic relationship might be expected to exist between them. If an intermediate such as XIII is a percursor of lycopodine, then biogenetically possible modifications could include a dinitrogeneous intermediate such as XIV.



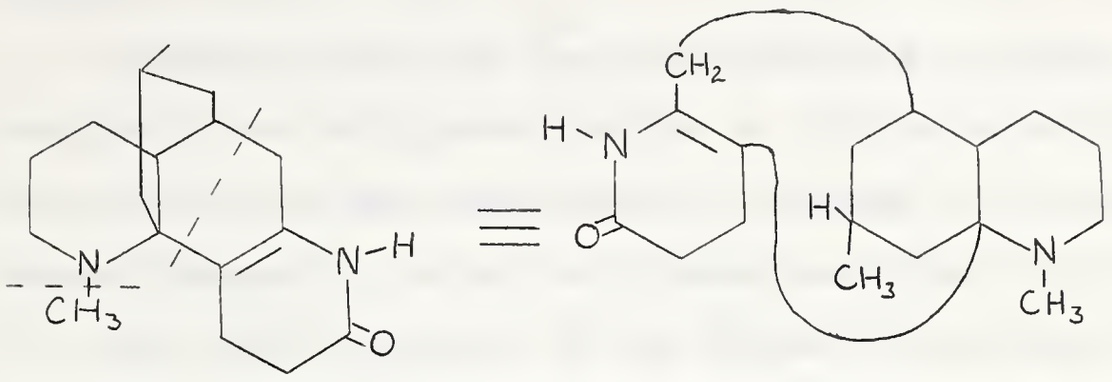
XIII



XIV

Lactam formation, and N-methylation provides a structure

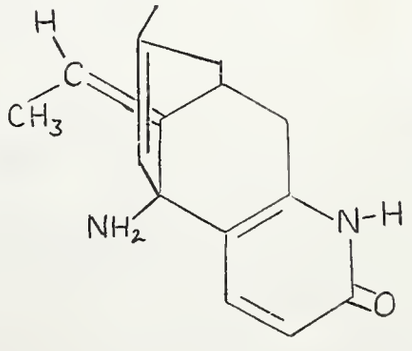
XV which incorporates all of the characteristics of α-obscurine previously presented.



XV

The dashed lines in Figure XV indicate the bond cleavages necessary to produce the dehydrogenation fragments. β-Obscurine would then be the corresponding 2-pyridone.

Since the existence of the possible intermediates XIII or XIV has not been demonstrated, the structures which rest upon arguments which utilize them cannot be considered to be rigorously proved. A degree of support for these structures was supplied by the structure elucidation of selagine (XVI) (44) a constituent of Lycopodium selago L.

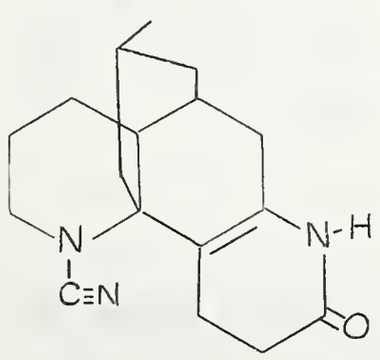


XVI

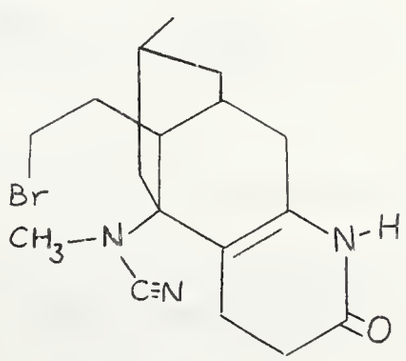
Selagine also gives 6-methyl-2-pyridone on dehydrogenation (44).

Rigorous proof for the structures was obtained by chemically relating α-obscurine to dihydrolycopodine. The method used was essentially a reversal of the suggested biogenetic route from lycopodine to α-obscurine.

The first objective in the series of reactions leading to dihydrolycopodine was the removal of the methyl group from the basic nitrogen of α-obscurine. When α-obscurine was subjected to the von Braun cyanogen bromide reaction (47), it was possible to isolate a neutral glassy material in 45% yield. The infrared absorption spectrum showed a sharp band at 2200 cm^{-1} indicative of a cyano group. Elution chromatography and treatment with various solvents failed to yield crystalline material. Analysis of a distilled sample indicated that the material contained bromine (2.9%), indicating a mixture of the desired demethylated compound XVII (ca. 85%) and ring cleaved compound XVIII (ca. 15%).



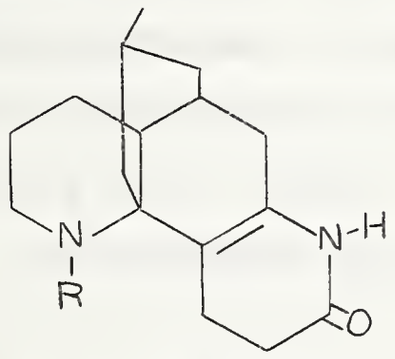
XVII



XVIII

Since definitive results were desired at this point, we decided to try nitrous acid as a demethylating agent, since this method had been so successful in removing the N-ethyl group in delpheline (34).

Treatment of α-obscurine with nitrous acid in aqueous acetic acid resulted in a 40% yield of a neutral compound ($C_{16}H_{23}O_2N_3$ M.P. 271-273°) showing absorption in the infrared (Nujol) at 3220, 3100 (>N-H) and 1708, 1683, 1645 cm^{-1} (dihydropyridone), and in the ultraviolet at 240 mμ (log ϵ = 3.92). The ultraviolet absorption spectrum is presumably the result of the coalescence of the absorption bands of the dihydropyridone system (λ max 255) and the N-nitroso system (N-nitrosodiethylamine shows an absorption maximum at 233 mμ with log ϵ = 3.87 (51)). The observed characteristics are consistent with formulation XIXa.



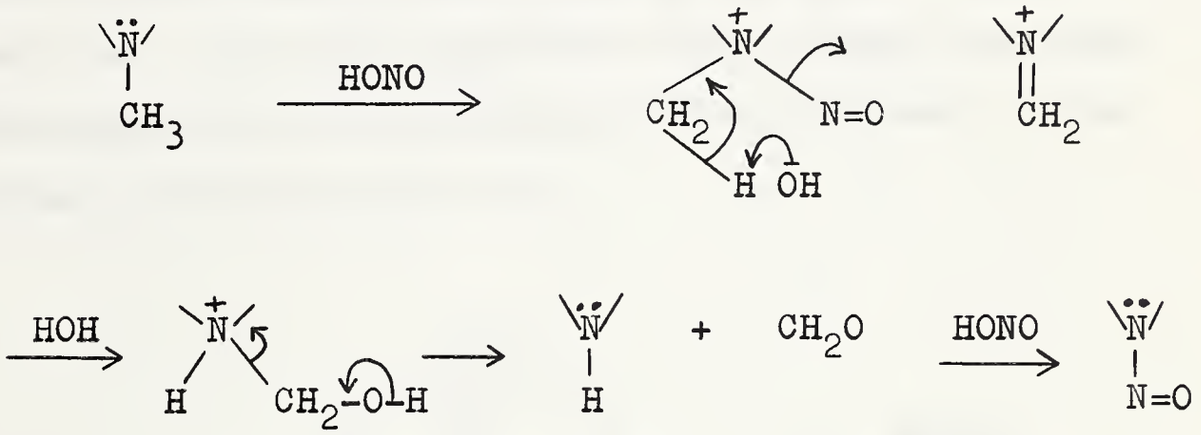
XIX a R = -N=O

XIX b R = H

The basic material recovered from the reaction solution proved to be unchanged α-obscurine.

The manner in which the demethylation takes place is

apparently not a simple displacement reaction since Halliday and Reade (52) showed that the treatment of p-nitro-N,N-dimethylaniline with nitrous acid provides formaldehyde along with the expected N-nitroso compound. A possible reaction sequence incorporating this feature is outlined below.



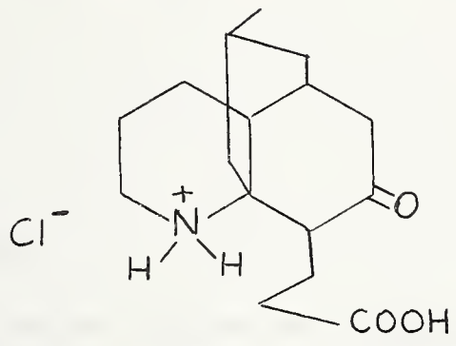
Hydrolysis of de-N-methyl-N-nitroso- α-obscurine (XIXa) with dilute hydrochloric acid gave a basic compound ($C_{16}H_{24}ON_2$, M.P. 266 - 268°) showing absorption in the infrared at 3270, 3200 (N-H) and 1698, 1680, 1642 cm^{-1} (dihydropyridone). The ultraviolet spectrum showed the typical absorption of the dihydropyridone system (λ max 255 mμ, logϵ = 3.8).

The available data is consistent with the formulation XIXb.

At about this time, it was found that de-N-methyl-α-obscurine occurs naturally in Lycopodium clavatum Linn (28). Methylation of the natural demethyl compound with

formic acid - formaldehyde (28) afforded ∝-obscurine.

De-N-methyl-∝-obscurine was refluxed with 12N hydrochloric acid until the absorption in the ultraviolet (λ max 255 mμ) disappeared (108 hours). The light brown foam which was obtained by evaporation of the solvent showed broad bands in the 3500 - 2300 cm^{-1} and 1700 - 1580 cm^{-1} regions of the infrared spectrum, consistent with the presence of the functional groups shown in structure XX (35).



+ NH_4Cl

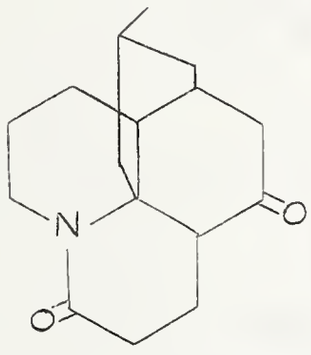
XX

Attempts to isolate compound XX or the corresponding methyl ester were unsuccessful. Ammonium chloride, an expected product of the reaction, was isolated by trituration of the foamy residue with acetone. The infrared spectrum of the crystalline residue was identical to that of authentic ammonium chloride.

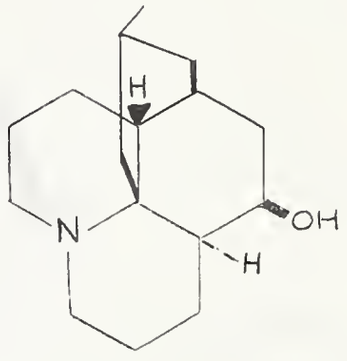
The cyclization involving the nitrogen and the carboxylic acid group does not take place with facility, since at no time was a neutral compound obtained directly from the hydrolysis solution.

Treatment of the crude hydrolysis product with

dicyclohexylcarbodiimide (36,37) in pyridine gave a neutral compound ($C_{16}H_{23}O_2N$, M.P. 177 - 180°) in 24% yield (after chromatography) which showed absorption in the infrared at 1700 cm^{-1} and 1626 cm^{-1}. The infrared spectrum was almost identical to the infrared spectrum of the keto-lactam XXI previously prepared from lycopodine (38).



XXI

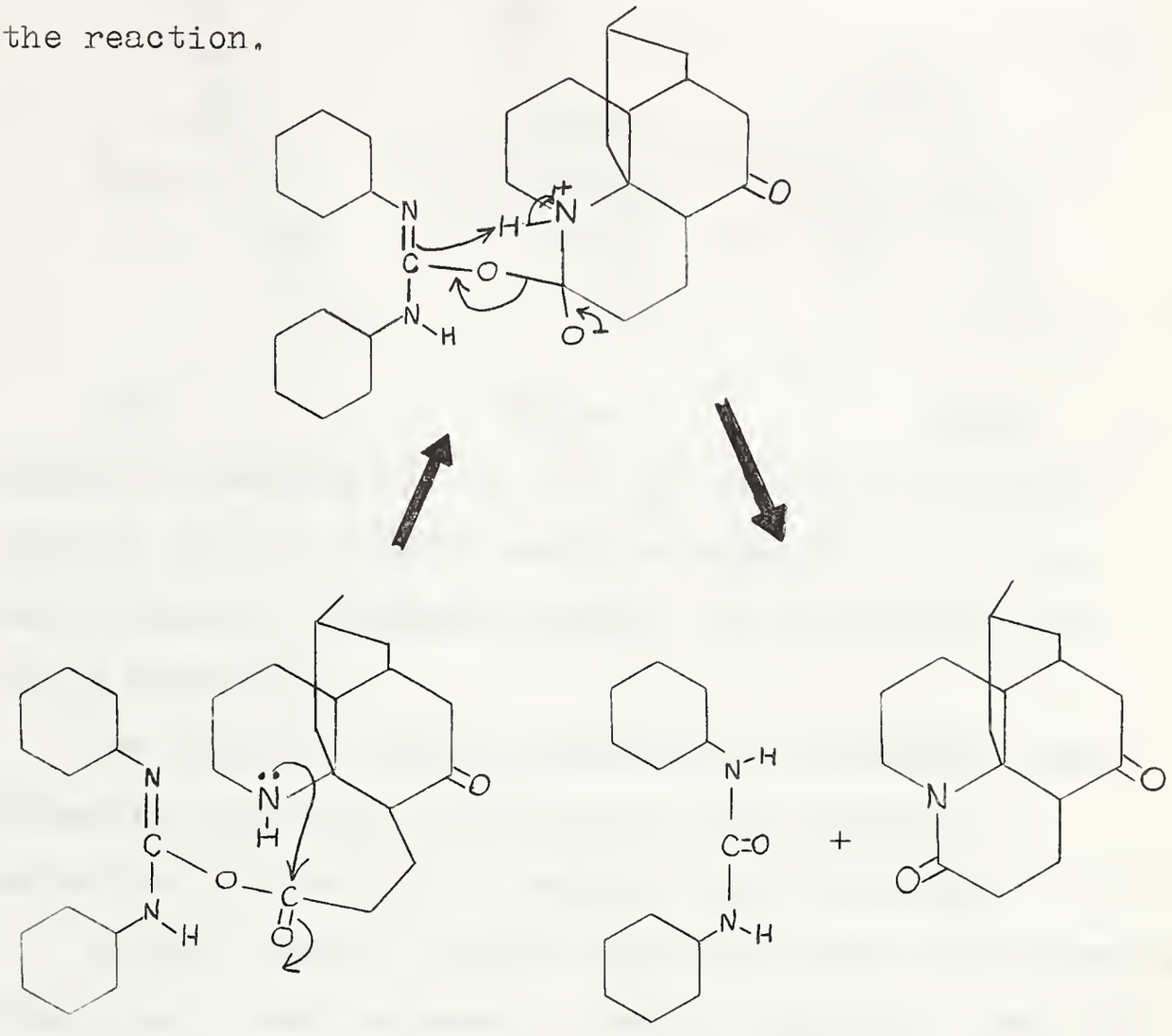


XXIa

Reduction of XXI with $LiAlH_4$ in diglyme gave dihydrolycopodine (XXIa), identical (M.P., M.M.P., infrared spectrum, and optical rotation) to authentic material prepared by the reduction of lycopodine with $LiAlH_4$. This transformation of α-obscurine into dihydrolycopodine fully supports the structural assignments previously made for α- and β-obscurine.

A possible mechanism for the cyclization of XX to XXI is outlined below. The first step presumably is the protonation of one of the basic nitrogens of the carbodiimide by the carboxylic acid proton and subsequent attack of the carboxylate anion on the positively charged carbon of the protonated carbodiimide system to give

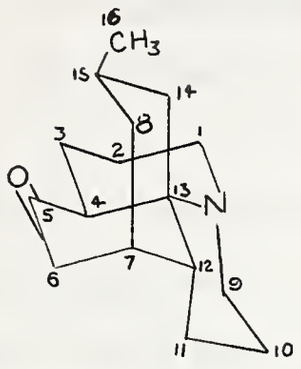
intermediate (a). Addition of the basic nitrogen to the carboxyl followed by loss of dicyclohexylurea completes the reaction.



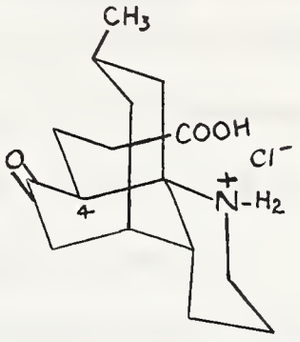
a

The relative and absolute stereochemistry of α- and β-obscurine may be assigned on the basis of this result since both the relative (39) and absolute stereochemistry (40) of lycopodine has been determined. Lycopodine is represented by structure XXII and numbered according to a proposal by Wiesner (40a). The intermediate XX may be written as XXIIa and thus the obscurines as structure XXIII. It should be pointed out that the route from α-obscurine to dihydrolycopodine affects only carbon

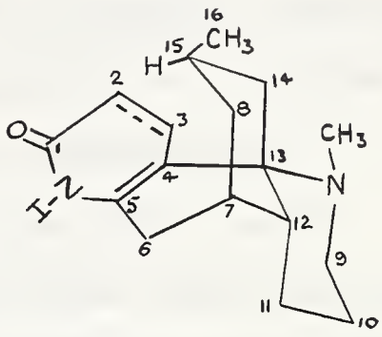
atoms 4 and 5 of α-obscurine and does not disturb the



XXII



XXIIa



XXIII

asymmetric carbons (7, 12, 13, 15) and the substituent attached to C-4 of XXIIa would be expected to take up the equatorial conformation under the acid conditions of the hydrolysis.

The N-methyl group is assigned the presumably more favorable equatorial conformation (41) although no definitive information is available on this point.

At this point it can be seen that these dinitrogeneous alkaloids do not represent a dramatic departure from the lycopodine system. A biogenetic hypothesis for these alkaloids will be presented in detail later (See page 108).

3. THE STRUCTURE OF LYCODINE.

Lycodine was first isolated from Lycopodium annotinum Linn. in 1958 by Anet and Eves (20) who utilized a combination of countercurrent distribution and elution chromatography to effect the separation and purification of this alkaloid.

The lycodine used in our structural investigation was

obtained from two Lycopodium species; Lycopodium annotinum Linn. and Lycopodium obscurum Linn.

The isolation of lycodine from Lycopodium annotinum Linn. was carried out by rechromatography of fractions eluted by benzene and ether from a chromatogram of "annotinine free" crude alkaloid. Fractions which showed the typical ultraviolet absorption characteristics of lycodine (λ max 268, 276) were combined and rechromatographed. Elution with benzene-ether in various proportions eventually provided fractions which contained lycodine in sufficient concentration to allow crystallization from pentane solution. Lycodine in the presence of high concentrations of other alkaloids seems to elute slowly over a relatively wide range of solvent polarity.

The crude alkaloid obtained from Lycopodium obscurum Linn. by the method of Manske and Marion (23) was chromatographed over basic alumina. The material eluted from the column with chloroform was rechromatographed over alumina. Elution with benzene provided lycopodine, elution with ether provided a mixture of lycopodine and lycodine from which lycodine could be obtained by crystallization from pentane. Elution with ether-methylene chloride (1:1) provided a mixture of lycodine and unidentified carbonyl containing compounds. The mother liquors from which crystalline lycodine was obtained were combined with the last fraction and treated with $LiAlH_4$. Chromatography of the crude reduction product and elution with benzene-ether provided further crystalline

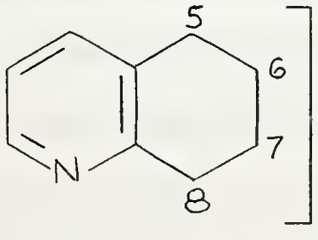
lycodine. Lycodine had not been previously isolated from Lycopodium obscurum Linn.

The results of an initial structural study carried out on lycodine by Anet and Eves (20) indicated a $C_{17}H_{24}N_2$ molecular formula for the alkaloid. The existence of a tertiary C-methyl group in the compound was suggested by the production of volatile acid on Kuhn-Roth oxidation and a sharp three proton signal at high field in the N.M.R. spectrum. The infrared spectrum showed >N-H stretching at 3270 cm^{-1} (CCl_4) and absorption at 1580 cm^{-1} (Nujol) indicating a pyridine ring system (35).

The secondary nature of one of the nitrogen atoms was supported by acetylation of the compound to a monoacetyl derivative.

The pyridine ring was shown to be substituted at the 2 and 3 positions by comparison of the N.M.R. spectra of lycodine, 2,3-lutidine, and pyridine. The pyridine ring was further shown to be a part of a 5,6,7,8-tetrahydroquinoline system by the comparison of the ultraviolet absorption spectra of lycodine, 2,3-lutidine, 2,3-trimethylene pyridine, 2,3-tetramethylene pyridine, and 2,3-pentamethylene pyridine.

The net result of this structural study was the proposal of the partial structure XXIIIa(20).



+ >N-H (not attached to C-5 or C-8)

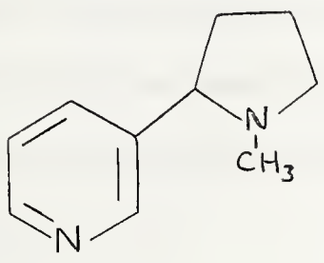
$-CH_3$ (attached to a tertiary carbon)

7 carbon and 17 hydrogen atoms in two additional rings.

XXIIIa

The existence of two additional rings was indicated since no other unsaturations were detectable in the compound by N.M.R., infrared, or ultraviolet spectroscopy.

The prohibition about the attachment of the secondary nitrogen rested upon a comparison of the relative basicities of lycodine and nicotine (XXIV).



Nicotine pKa 3.35, 7.70

Lycodine pKa 3.97, 8.08

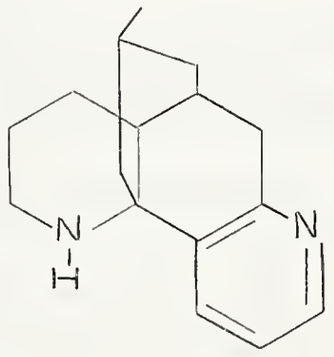
XXIV

The greater basicity of the pyridine nitrogen in lycodine as compared to the basicity of the pyridine nitrogen in nicotine was explained by the assumption that the saturated nitrogen is further removed from the pyridine ring in lycodine than it is in nicotine.

Anet and Eves (20) suggested that there might be a close relationship between lycodine and β-obscurine, and as mentioned previously, attempted to clarify this by the acetylation of β-obscurine. The failure of β-obscurine to undergo acetylation seemed to preclude a simple relationship between the two compounds since it was not known at that time that the basic nitrogen of β-obscurine carried a methyl group (25).

During the structural development of α- and β-obscurine,

it became evident that the C-methyl group in lycodine was not necessarily tertiary since the secondary C-methyl system in β-obscurine did not show a split signal in the N.M.R. spectrum, although the existence of the secondary system in β-obscurine was shown by the unambiguous interconversion of α- and β-obscurine. In addition, when the environment of the basic nitrogen of β-obscurine was clarified, and it was shown to carry an N-methyl group, then an attractive possibility for the structure of lycodine became the de-N-methyl-pyridine analogue of β-obscurine, i.e. Figure XXV.



XXV

The first difficulty which arose in the consideration of this structure was the published molecular formula. Structure XXV is a $C_{16}H_{22}N_2$ compound while Anet and Eves (20) had reported a molecular formula ($C_{17}H_{24}N_2$) for lycodine. The analytical results which were available at the time are tabulated on the following page:

LYCODINE.DIPICRATE: (Reference 20).

FOUND %			CALCULATED %					
			$C_{17}H_{24}N_2.2\ C_6H_3O_7N_3$			$C_{16}H_{22}N_2.2\ C_6H_3O_7N_3$		
C	H	N	C	H	N	C	H	N
48.20 48.47	4.02 4.03	15.91 15.64	48.74	4.23	15.68	47.99	4.03	16.00

N-ACETYLLYCODINE.PICRATE: (Reference 20).

FOUND %			CALCULATED %					
			$C_{19}H_{26}ON_2.C_6H_3O_7N_3$			$C_{18}H_{24}ON_2.C_6H_3O_7N_3$		
C	H	N	C	H	N	C	H	N
56.58	5.39	13.69	56.92	5.54	13.28	56.14	5.30	13.64

LYCODINE: (Reference 20).

FOUND %			CALCULATED %					
			$C_{17}H_{24}N_2$			$C_{16}H_{22}N_2$		
C	H	N	C	H	N	C	H	N
79.50 79.46	9.44	11.16	79.64	9.43	10.93	79.29	9.15	11.56

LYCODINE: (This work).

FOUND %			CALCULATED %					
			$C_{17}H_{24}N_2$			$C_{16}H_{22}N_2$		
C	H	N	C	H	N	C	H	N
79.49	9.13	11.61	79.64	9.43	10.93	79.29	9.15	11.56
79.33	9.09	11.47						

Examination of the figures for the first three analyses show that it would be very difficult to distinguish between the $C_{16}H_{22}N_2$ and $C_{17}H_{24}N_2$ formulations. The final analysis, which was carried out in the present work, supports the $C_{16}H_{22}N_2$ formulation better than the $C_{17}H_{24}N_2$ formulation. The result is clearly shown in the following table which compares the amount by which the calculated values for the two formulae under consideration differ from the average found value for each element.

	Average Found %	Calc. $C_{17}H_{24}N_2$	△	Calc. $C_{16}H_{22}N_2$	△
C	79.41	79.64	.23	79.29	.12
H	9.11	9.43	.32	9.15	.04
N	11.54	10.93	.61	11.56	.02

Structure XXV also places the secondary nitrogen at carbon atom 5 (as designated in part structure XXIIIa,

contrary to the original proposal. We felt that the difference in the relative basicities of the pyridine nitrogens in nicotine and lycodine could be interpreted in two ways. Anet and Eves suggested that the basicity of the pyridine nitrogen in nicotine was suppressed (as compared to the basicity of the pyridine nitrogen in lycodine) because of the proximity of the saturated nitrogen in nicotine. This interpretation required at least one additional carbon atom between the saturated nitrogen and the pyridine ring in lycodine. We felt that the observed differences could be interpreted rather as an enhancement of the basicity of pyridine nitrogen in lycodine (as compared to the pyridine nitrogen in nicotine) by the electropositive alkyl substituent (42) at the 2-position of the pyridine ring in lycodine. If this interpretation is accepted, structure XXV becomes consistent with the observed basicities.

Proof that structure XXV does indeed represent lycodine was obtained by the conversion of β-obscurine to lycodine and N-methyllycodine.

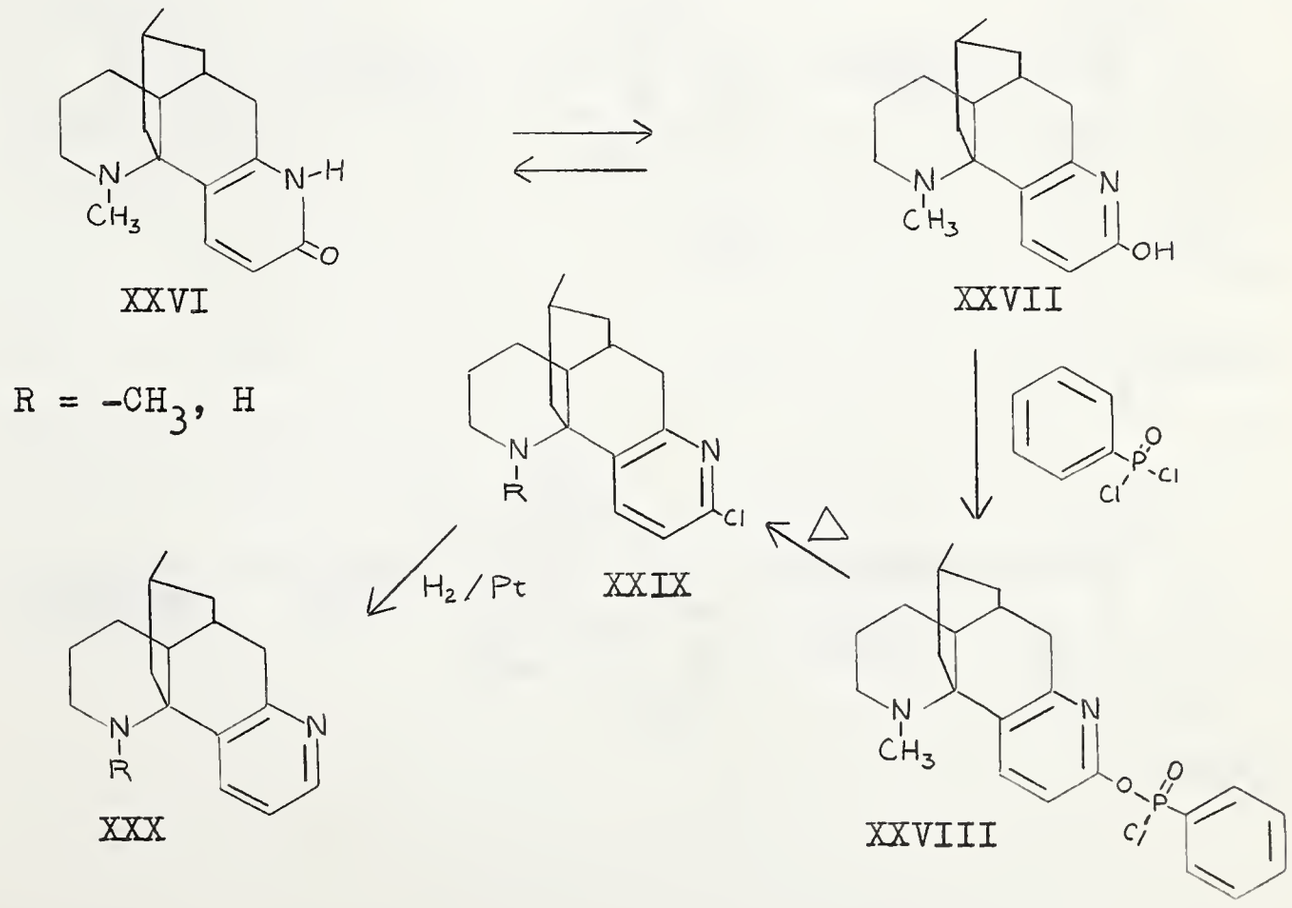
N-Methyllycodine (prepared by refluxing lycodine in a mixture of 98% formic acid - 40% formaldehyde solution) was thought to be the most convenient compound for comparison because it was anticipated that the N-methyl group of β-obscurine might be difficult to remove efficiently. (It was subsequently found that de-N-methyl-β-obscurine could be prepared by the same method as that used in the preparation of de-N-methyl-α-obscurine).

Conversion of a 2-pyridone to the corresponding pyridine is usually accomplished by the catalytic reduction (hydrogenolysis) of an intermediate 2-chloropyridine derived by the treatment of the 2-pyridone with a chlorinating reagent such as $POCl_3$ or PCl_5 (43). β-Obscurine was recovered unchanged from treatment with refluxing $POCl_3$, and from heating with mixtures of $POCl_3$ and PCl_5. The desired chloropyridine appeared to be formed by reacting a mixture of β-obscurine and PCl_5 in a sealed tube at elevated temperatures, however it was found that the intermediate could be obtained more conveniently by treatment of β-obscurine with hot (200°) phenylphosphonic dichloride (44).

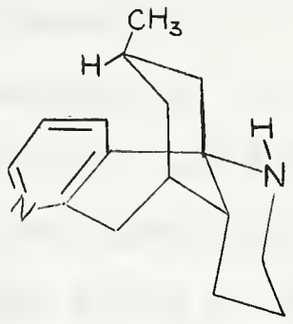
The oily basic material recovered from the reaction mixture showed absorption in the ultraviolet at 275 mμ and in the infrared (CCl_4) at 1565 cm^{-1} and 3330 cm^{-1}. The ultraviolet absorption band was in the same general region as the ultraviolet absorption bands in N-methyllycodine (268, 275 mμ). The infrared absorption at 1565 cm^{-1} appeared analogous to a band at 1574 cm^{-1} in the infrared spectrum of N-methyllycodine. The absorption in the infrared at 3330 cm^{-1} appeared at the time to be anomalous since no >N-H was expected in crude reaction product. No attempt was made to isolate or further characterize the crude product but it was reduced by shaking under hydrogen (50 p.s.i.) in glacial acetic acid in the presence of Adam's catalyst.

The basic product of the reduction was separated into

two crystalline compounds by elution chromatography over basic alumina. The first compound (38% from β-obscurine) was shown to be N-methyl-lycodine identical to authentic material (comparison of infrared and ultraviolet spectra, melting point, and undepressed mixed melting point). The second was shown to be lycodine (20% from β-obscurine) identical to authentic lycodine by the same criteria used to establish the identity of the first compound. The isolation of lycodine explains the absorption band at 3330 cm^{-1} (N-H) in the infrared spectrum of the crude chloropyridines. The lycodine apparently arises by elimination of the methyl group from β-obscurine (or the intermediate chloropyridine) during the reaction. The reaction sequence is outlined by structures XXVI to XXX.

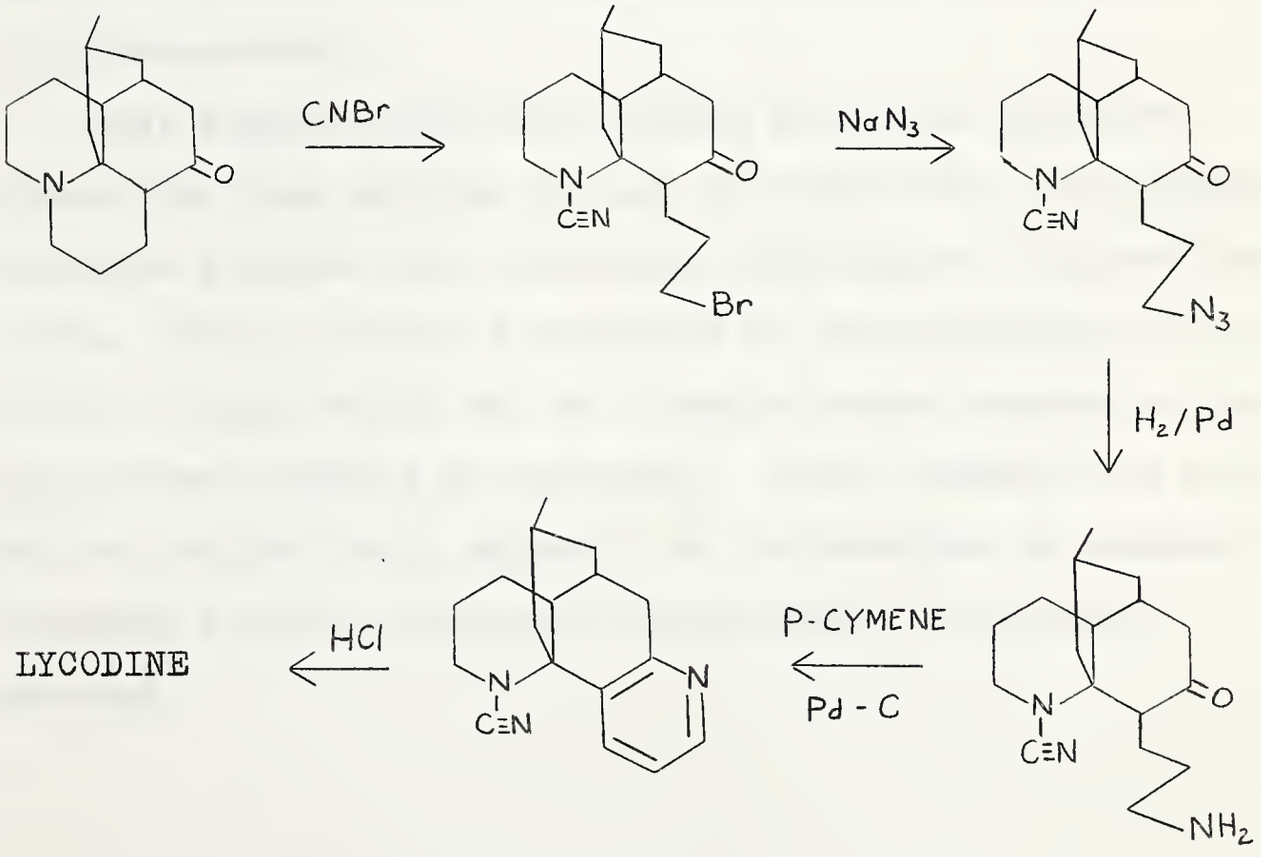


On the basis of the stereochemistry assigned to α- and β-obscurine in the previous section, lycodine may be sterically represented by XXXI.



XXXI

Anet and Rao (48) carried out the transformation of lycopodine (XII) to lycodine (XXV) by the following series of reactions.



Chronologically this work was carried out while we were engaged in the transformation of α-obscurine to dihydrolycopodine and thus constituted additional early support for the structures of α-obscurine, β-obscurine and lycodine.

The anomalous behaviour of the -C-methyl groups of β-obscurine and lycodine in the N.M.R. has been discussed by Anet (46). Stereostructures XXIII and XXXI place the methine proton above the plane of the aromatic ring in lycodine and above the plane of the 2-pyridone ring of β-obscurine. 2-Pyridone systems display some degree of aromatic character (23,43) and it has been shown that protons which lie above or below the plane of an aromatic ring (which is thus capable of carrying a ring current), undergo a diamagnetic shift and thus resonate at a higher field than normal.

Anet suggests that the methine proton in these two systems has been shifted to such an extent that the adjacent methylene protons cause extensive splitting of this methine proton. This in turn is reflected in the splitting of the methyl protons (which act as a single proton because of the equivalence obtained by rotation). Thus, instead of a well defined doublet being apparent in the spectrum, a somewhat broadened singlet composed of many unresolved lines is observed.

EXPERIMENTAL: (α -OBSCURINE, β -OBSCURINE AND LYCODINE)

Analyses were performed by Pascher Microanalytisches Laboratorium, Bonn, West Germany, and C. Daessle, Montreal, Quebec.

Melting points were obtained on a Fisher-Johns Hot Stage Melting Point Apparatus and are uncorrected.

The infrared spectra were recorded by Perkin Elmer Infracord, and models 21 and 221 G Recording Spectrometers using sodium chloride cells or plates.

Ultraviolet spectra were obtained with a Cary Model 14 Recording spectrometer (1 cm guartz cells, 95% Ethanol solution).

1. ANALYSIS OF α-OBSCURINE:

α-Obscurine was isolated from the crude alkaloid of Lycopodium annotinum Linn. by elution chromatography as outlined in the foregoing discussion. Crystallization from acetone-methanol to constant melting point (282-283°) provided the analytical sample which was sublimed (280°/ 0.5 mm) for analysis. (FOUND: C, 74.43, 74.61; H, 9.51, 9.40; N, 10.27; $N\text{-}CH_3$, 5.49; $C\text{-}CH_3$, 3.03. CALCULATED FOR $C_{17}H_{26}ON_2$: C, 74.41; H, 9.55; N, 10.21; 1- $N\text{-}CH_3$, 5.48; 1- $C\text{-}CH_3$, 5.48%).

2. ANALYSIS OF β-OBSCURINE:

β-Obscurine was isolated from the crude alkaloid of Lycopodium annotinum Linn. by elution chromatography as described in the foregoing discussion. Crystallization from acetone-methanol to constant melting point (317-318°) provided the analytical sample. (FOUND: C, 74.88, 74.78; H, 8.74, 8.82; N, 10.13; $N\text{-}CH_3$, 5.21, 5.42. CALCULATED FOR $C_{17}H_{24}ON_2$: C, 74.96; H, 8.88; N, 10.29; $N\text{-}CH_3$, 5.48%).

3. β-OBSCURINE FROM α-OBSCURINE:

α-Obscurine (180 mg), N-bromosuccinimide (235 mg), and benzoyl peroxide (1 mg) were refluxed in CCl_4 (30 ml) under illumination by an ordinary 100 watt incandescent lamp for 1 hr. The reaction mixture was diluted with $CHCl_3$, washed with 2N NaOH, dried and evaporated to yield a light brown foam (100 mg). Chromatography over alumina

(5 g) gave, on elution with CH_2Cl_2:$CHCl_3$ (1:1), unreacted α-obscurine (72 mg). Elution with $CHCl_3$MeOH (39:1) and crystallization from acetone-methanol yielded β-obscurine (20 mg) identical with an authentic sample in m.p., mixed m.p., and infrared spectrum.

4. α-OBSCURINE FROM β-OBSCURINE:

A solution of β-obscurine (31 mg) in dry tetrahydrofuran (100 ml) was added during 1/2 hour to a vigorously stirred solution of lithium (0.1 g) in ammonia (40 ml). The solution was stirred for a further 2½ hours, then the reaction mixture was evaporated to dryness. The residue was dissolved in aqueous HCl, washed with chloroform, basified, and extracted with chloroform. Evaporation of the chloroform left a solid residue which was chromatographed over alumina (1 g). Elution with chloroform and crystallization from methanol gave α-obscurine (20 mg), m.p. 285 - 287° (uncorrected). The mixture melting point with authentic α-obscurine was undepressed, and the infrared spectra of the two were identical.

Further elution of the column with chloroform-methanol (4:1) gave a fraction (7 mg) whose spectral properties were identical with those of β-obscurine.

5. REACTION OF α-OBSCURINE WITH CYANOGEN BROMIDE:

Preliminary experiments, using cyanogen bromide under reaction conditions which previously had been successful in obtaining nitrogen-carbon bond cleavage in lycopodine (49,50),

showed α-obscurine to be much more stable towards this reagent than lycopodine. Reaction conditions investigated ranged from overnight treatment of α-obscurine with CNBr in $CHCl_3$ at room temperature, to refluxing a $CHCl_3$ solution of α-obscurine and CNBr for 48 hours. Yields of crude neutral material were in the 15-20% range. No better results were obtained by treatment of α-obscurine with CNBr in a sealed tube. The highest yield of cyanamide was obtained in the following manner: α-Obscurine (104 mg) and cyanogen bromide (1.2 g) were dissolved in $CHCl_3$ (5 ml). Sodium carbonate (0.07 g) was added and the mixture refluxed for 21 hr. The solvent and the excess cyanogen bromide were removed under reduced pressure and the residue distributed between chloroform and dilute HCl. The aqueous layer was separated, made basic with dilute NH_4OH, and extracted with $CHCl_3$ to yield unreacted α-obscurine (50 mg).

The chloroform layer was washed with water, dried ($MgSO_4$), and evaporated to give a pale yellow glass (48 mg) which showed strong absorption at 2200 cm^{-1} in the infrared. The combined neutral fractions (360 mg) from several reactions were chromatographed over alumina (60 g). Elution with CH_2Cl_2:MeOH (99:1) gave 240 mg of a colourless viscous oil which could not be induced to crystallize. Further chromatography failed to effect a separation (as judged by inspection of the infrared spectra of the various fractions). The material purified in this manner showed bands in the infrared at 3420 cm^{-1} (N-H), 2200 cm^{-1} (-C≡N) and 1675 cm^{-1} (dihydropyridone) and exhibited

a maximum in the ultraviolet at 255 mμ. A portion was distilled for analysis. (FOUND: C, 69.15; H, 8.16; N, 13.09; Br, 2.96. $C_{17}H_{26}ON_3$ requires: C, 71.55; H, 8.12; N, 14.72. $C_{17}H_{26}ON_3Br$ requires: C, 56.84; H, 6.89; N, 11.05; Br, 21.01). The found values agree reasonably well with a mixture of XVII and XVIII (17:3).

6. DE-N-METHYL-N-NITROSO-α-OBSCURINE (XIXa):

A solution of α-obscurine (204 mg) in 25% aqueous acetic acid (8 ml) was combined with 60% aqueous sodium nitrite (2 ml) and the resulting solution kept at room temperature for 19 hours. Dilute hydrochloric acid was then added and the resulting solution extracted with $CHCl_3$. The $CHCl_3$ extract was washed with dilute sodium carbonate and then water, dried, and evaporated to yield 81 mg crude, crystalline, de-N-methyl-N-nitroso-α-obscurine, m.p. 260-265°, suitable for the following step. The analytical sample was prepared by recrystallization from ethyl acetate-acetone, m.p. 271-273°. Infrared spectrum (nujol): ν max 3220, 3120 (NH), 1708 sh, 1683 s, 1645 m cm^{-1}. Ultraviolet spectrum, λ max 240 mμ ($\log \epsilon = 3.92$). (FOUND: C, 66.61, 66.08; H, 8.18, 7.95; O, 11.04; N, 14.42. $C_{16}H_{23}O_2N_3$ requires: C, 66.40; H, 8.01; O, 11.07; N, 14.52%).

The aqueous acid layer (above) yielded unchanged α-obscurine when worked up in the usual manner.

7. DE-N-METHYL-α-OBSCURINE (XIXb):

A solution of de-N-methyl-N-nitroso-α-obscurine (78 mg) in 2N HCl (35 ml) was refluxed for 2¼ hours. The solution was diluted with water, washed with $CHCl_3$ to remove unreacted neutral material, made alkaline with ammonium hydroxide solution, and extracted several times with $CHCl_3$. Removal of the $CHCl_3$ under reduced pressure gave 58 mg of crystalline material.

The combined crystalline material (540 mg) from several such runs was dissolved in benzene and chromatographed over alumina (15 g).

The fractions eluted with ether-dichloromethane (3:2) and dichloromethane and which displayed UV absorption at 255 mμ were combined and recrystallized from acetone to give colourless needles of de-N-methyl-α-obscurine, m.p. 266 - 268°. Infrared spectrum: ν max 3270, 3200 (N-H), 1698 m, 1680 s, 1642 (dihydropyridone). Ultraviolet spectrum: λ max 255 mμ ($\log \epsilon$ = 3.8). (FOUND: C, 73.77, 73.35; H, 9.89, 9.67; N, 10.81. $C_{16}H_{24}ON_2$ requires: C, 73.79; H, 9.29; N, 10.76%).

8. DE-N-METHYL-β-OBSCURINE:

β-Obscurine (260 mg) was treated with nitrous acid in the same manner as described for α-obscurine. The crystalline neutral product (78 mg, infrared bands at 3120, 1679, 1612, 1555 cm^{-1}, ultraviolet maxima at 230 and 315 mμ) was not further purified but was hydrolyzed to give de-N-

methyl-β-obscurine (52 mg) as colorless needles, m.p. 315° from methanol-acetone. Infrared spectrum (nujol): ν max 3330 (N-H), 1670, 1627, 1558 cm^{-1} (pyridone). Ultraviolet maxima at 230 and 315 mμ. (FOUND: C, 74.21, 74.30; H, 8.91, 8.84; N, 11.06%. $C_{16}H_{22}ON_2$ requires: C, 74.37; H, 8.58; N, 10.84%).

9. KETO-LACTAM XXI FROM DE-N-METHYL-α-OBSCURINE:

De-N-methyl-α-obscurine (149 mg) was refluxed in conc HCl (150 ml) until the 255 mμ chromophore had disappeared (108 hr). The cooled reaction solution was washed thoroughly with $CHCl_3$ and evaporated under reduced pressure to yield a light brown foam whose infrared spectrum exhibited broad bands in the 2300 - 3500 cm^{-1} and 1580 - 1700 cm^{-1} regions.

This material was dissolved in pyridine (100 ml), N,N' - dicyclohexylcarbodiimide (150 mg) added, and the resulting solution refluxed under nitrogen for 4 hours, then allowed to stand at room temperature for 18 hours. Removal of the solvent under reduced pressure yielded an oily residue which was distributed between $CHCl_3$ and dilute HCl. The chloroform layer was evaporated to yield a semi-solid residue which was triturated with CH_2Cl_2. The insoluble portion consisted of N,N' - dicyclohexylurea. The soluble portion was chromatographed over alumina (10 g). Elution with benzene, ether, and CH_2Cl_2 gave further dicyclohexylurea, but elution with $CHCl_3$ yielded an oily fraction (63 mg) which

crystallized after distillation (160 - 165°/0.5 mm). The solid material was rechromatographed over neutral alumina (3 g, activity I). Elution with CH_2Cl_2-$CHCl_3$ (1:1) gave 40 mg of crystalline material which was recrystallized from ether-Skellysolve B to give the keto-lactam XXI, m.p. 177 - 180°. The infrared spectrum (nujol), which showed carbonyl absorption at 1700 and 1626 cm^{-1}, was virtually identical with the infrared spectrum of the keto-lactam XXI prepared from lycopodine (38). (FOUND: C, 74.00; H, 8.64; O, 12.01. $C_{16}H_{23}O_2N$ requires: C, 73.52; H, 8.87; O, 12.24%).

10. DIHYDROLYCOPODINE FROM KETO-LACTAM (XXI):

Keto-lactam XXI (28 mg) was dissolved in diglyme (25 ml) containing a large excess of $LiAlH_4$ (200 mg). The reaction mixture was maintained at 85-92° for 4 hours, then the excess $LiAlH_4$ was destroyed by careful addition of wet ethyl acetate. Water and dilute HCl were added and the solution was washed with ether, then made basic with ammonium hydroxide and thoroughly extracted with $CHCl_3$. The dried extract was evaporated under reduced pressure to yield a crystalline residue (15 mg) which on recrystallization from ether afforded stout needles m.p. 166-169°, $(\alpha)_D^{25}$ - 33° (c, 0.56 in ethanol) identical in all respects (infrared spectrum, mixed m.p., optical rotation) to an authentic sample of dihydrolycopodine.

Formation of the perchlorate in methanol and recrystallization from methanol-ether provided colourless needles, m.p. 219-222°. The infrared spectrum of the perchlorate in

nujol was superimposable upon that of an authentic sample of dihydrolycopodine perchlorate and the mixed m.p. was undepressed.

11. N-METHYL LYCODINE FROM LYCODINE (XXV):

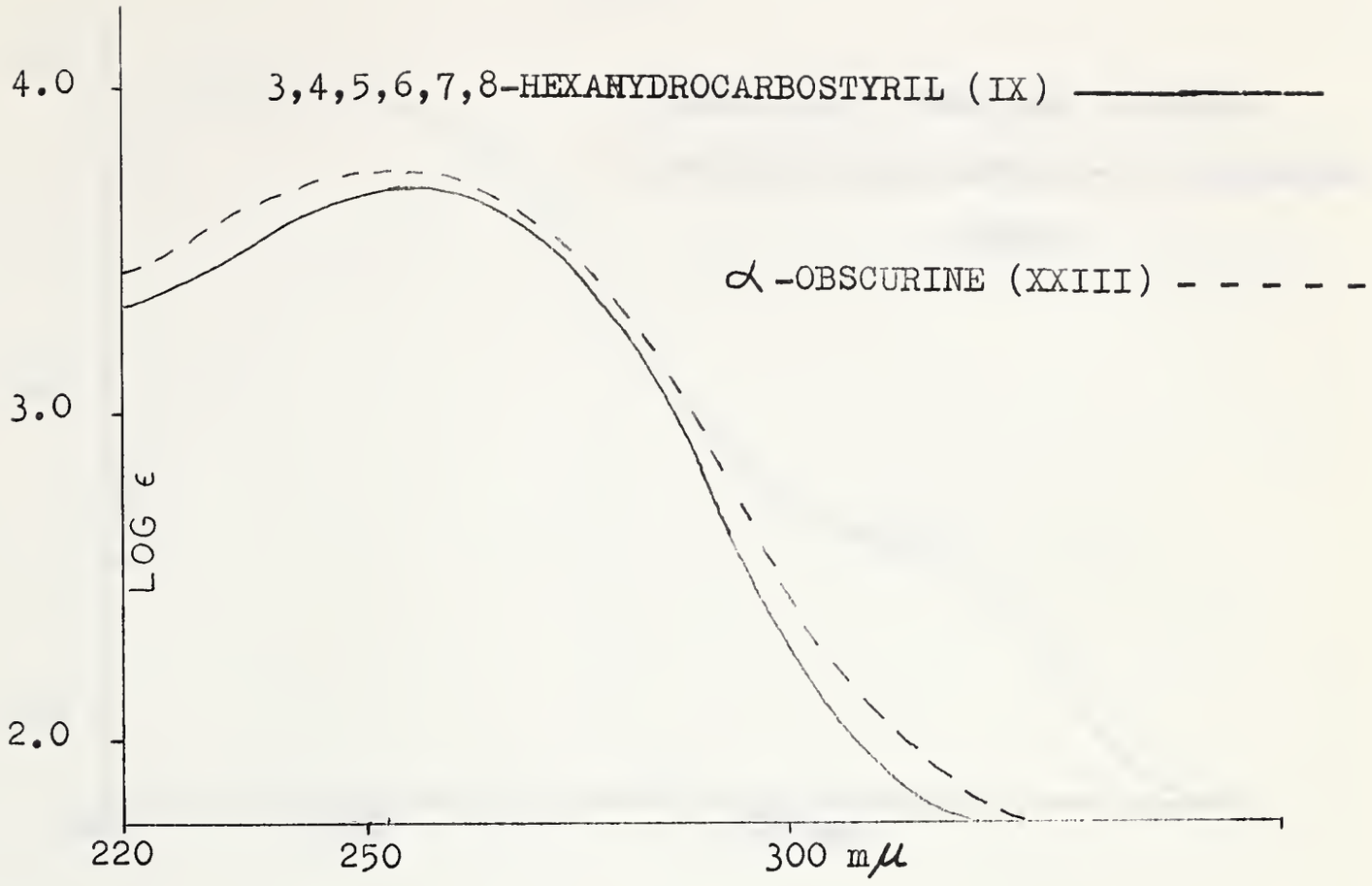
Lycodine (52 mg) was dissolved in 98% formic acid (0.25 ml) and formalin (0.25 ml) added. The solution was refluxed for 2 hours, then maintained at 50° for 12 hours. The reaction mixture was diluted with water (50 ml), basified with concentrated ammonium hydroxide, and extracted several times with chloroform. Evaporation of the dried chloroform extracts gave a pale yellow oil (0.05 g) which solidified on standing. Three recrystallizations from acetone gave N-methyl lycodine as colorless blocks (23 mg), m.p. 91-92°. Calculated for $C_{17}H_{24}N_2$: C, 79.63; H, 9.44; N, 10.93; one $N\text{-}CH_3$, 5.86%. Found: C, 79.61, 79.59; H, 9.36, 9.27; N, 11.25, 10.75; $N\text{-}CH_3$, 5.19%. Ultraviolet spectrum: λ max 268 mμ ($\log \epsilon$ = 3.61), shoulder at 275 mμ ($\log \epsilon$ = 3.49). Infrared spectrum (nujol): ν max 3040, 1574, 1472, 812, 737 cm^{-1} (pyridine ring).

12. N-METHYL LYCODINE AND LYCODINE (XXV) FROM β-OBSCURINE (XXIII):

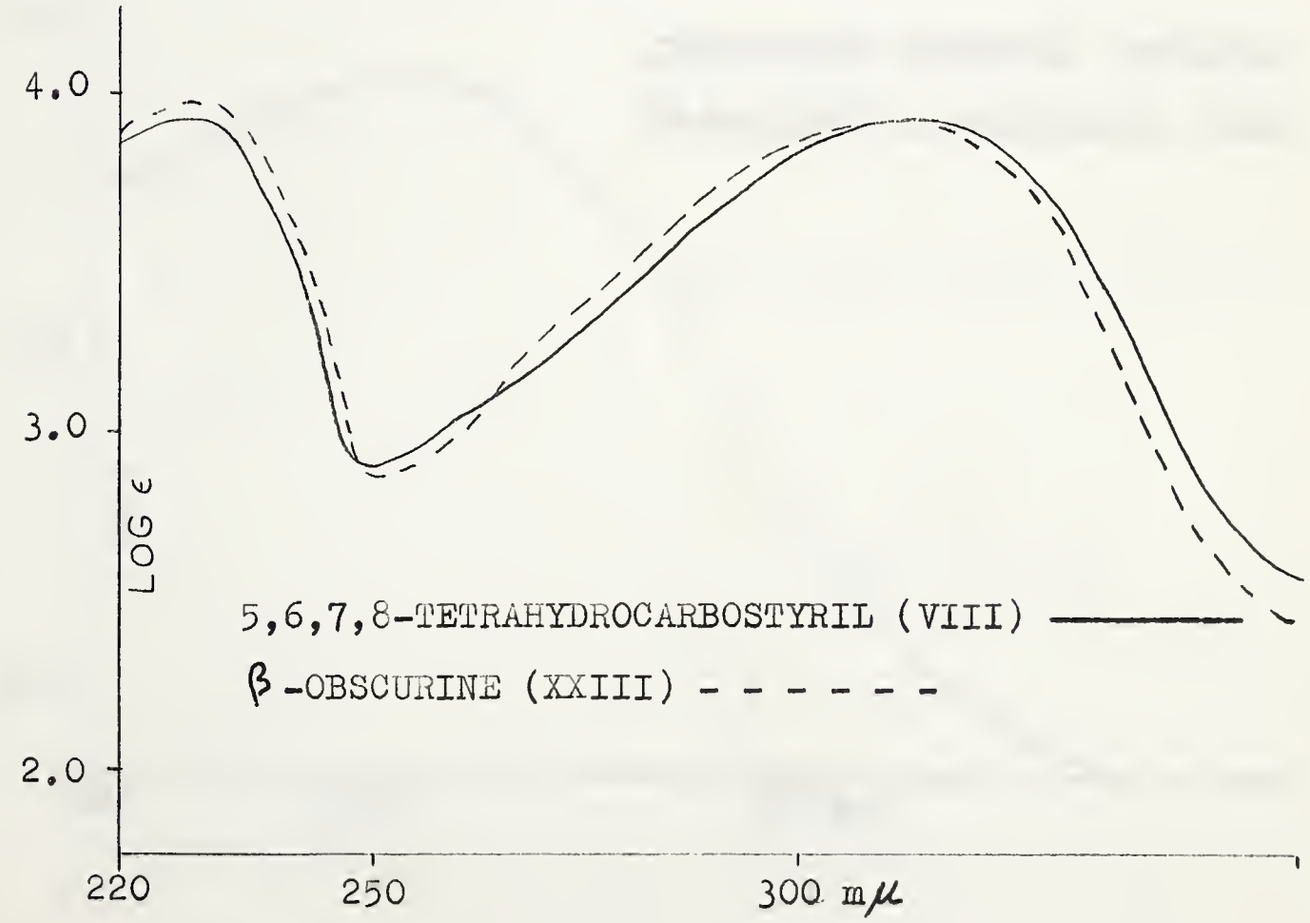
A solution of β-obscurine (168 mg) in phenylphosphonic dichloride (50 ml) was maintained at 200-210° for 45 minutes. The reaction solution was decomposed with ice and water and made basic by the addition of concentrated ammonium hydroxide. Chloroform extraction yielded the crude chloro compounds XXIX

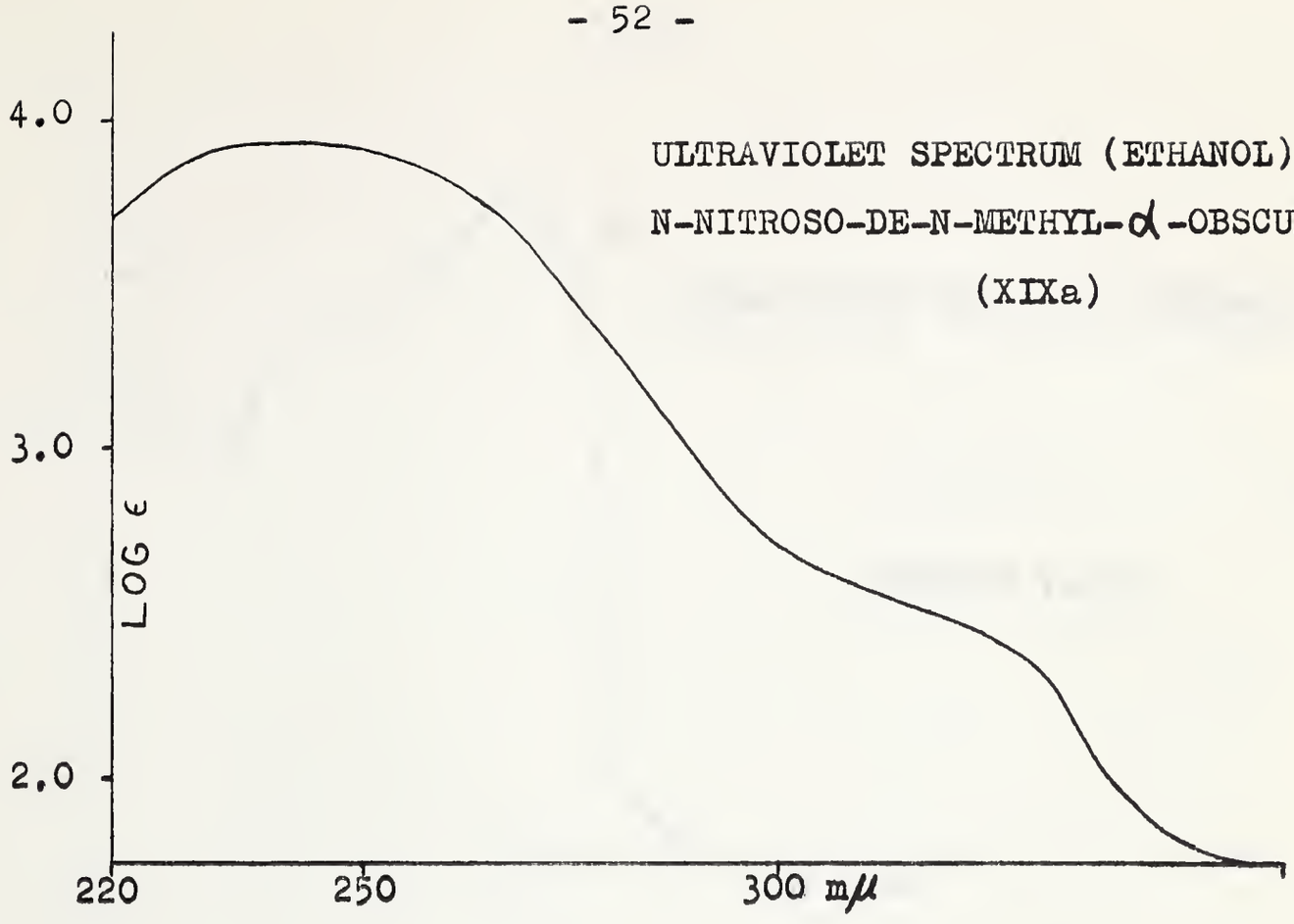
($R = CH_3$) and XXIX ($R = H$) as a pale yellow oil (0.16 g), ultraviolet maximum 275 mμ, infrared peaks at 3330 cm^{-1} (NH) and 1565 cm^{-1} (pyridine ring). The oil was dissolved in glacial acetic acid and shaken with hydrogen (50 p.s.i.) in the presence of Adam's catalyst (100 mg) for 3 hours. The catalyst was filtered and the filtrate concentrated, diluted with water, made basic with ammonium hydroxide, and extracted thoroughly with chloroform. Removal of the chloroform left a viscous yellow oil (0.12 g) which was chromatographed over basic alumina (3 g). Elution with benzene and crystallization from acetone gave N-methyl lycodine (65 mg), m.p. 91-92°. This did not depress the melting point of the N-methyl compound prepared from lycodine and their infrared spectra were identical.

Elution of the column with ether yielded a fraction (35 mg) which crystallized from acetone as colorless blocks, m.p. 115-116°. The infrared spectrum was identical with that of lycodine and a mixture melting point was undepressed.

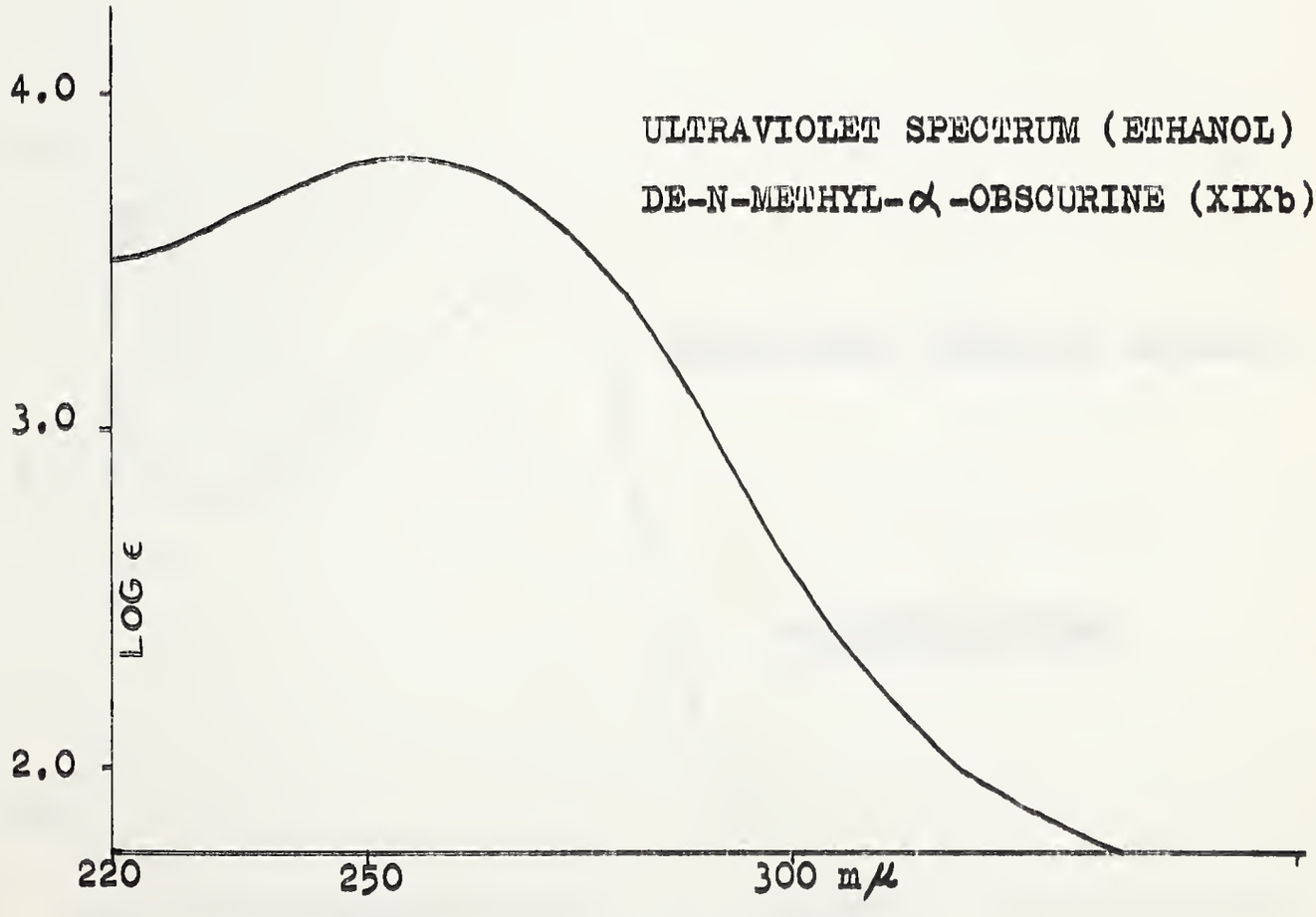


ULTRAVIOLET ABSORPTION SPECTRA (ETHANOL)

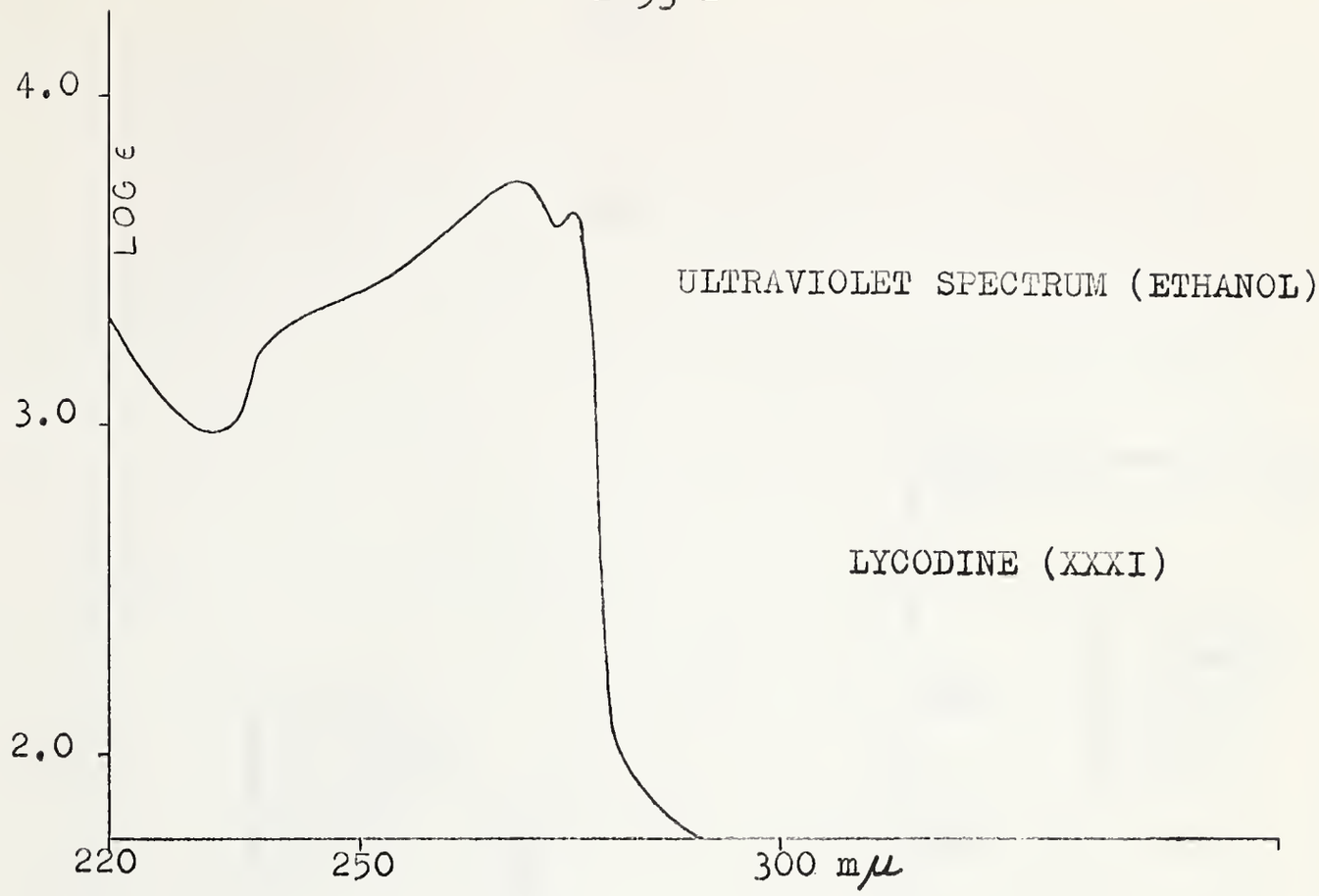




ULTRAVIOLET SPECTRUM (ETHANOL)
N-NITROSO-DE-N-METHYL-α-OBSCURINE
(XIXa)

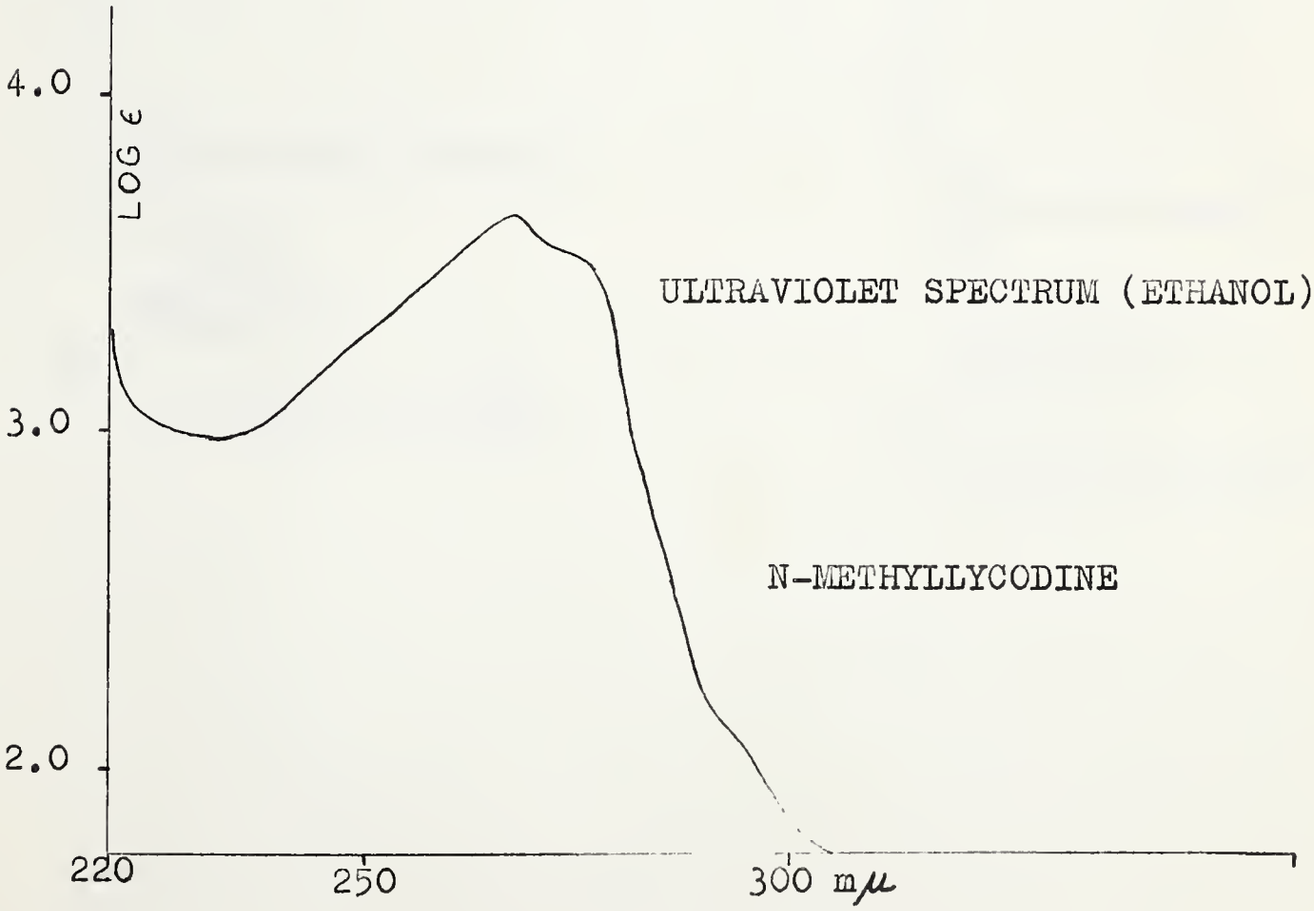


ULTRAVIOLET SPECTRUM (ETHANOL)
DE-N-METHYL-α-OBSCURINE (XIXb)



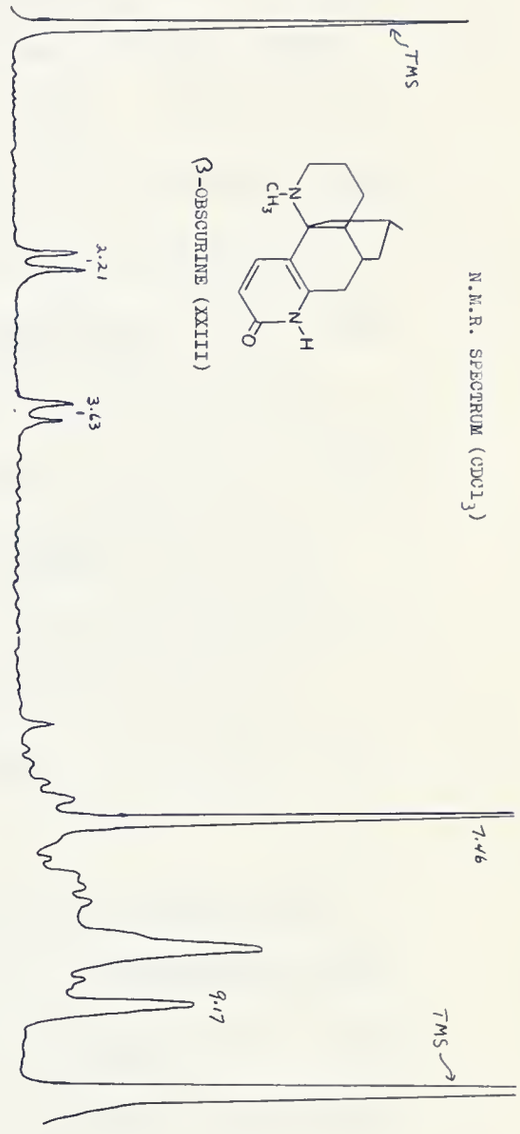
ULTRAVIOLET SPECTRUM (ETHANOL)

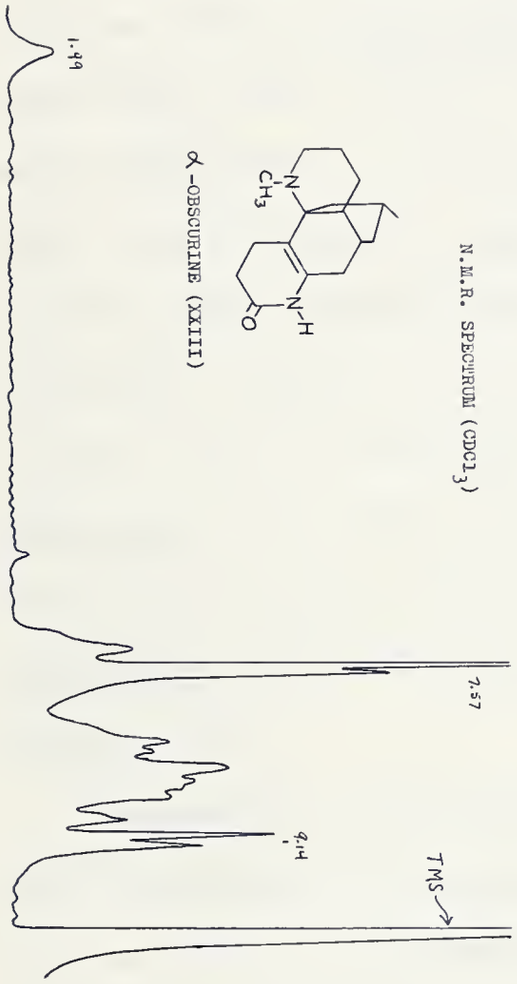
LYCODINE (XXXI)



ULTRAVIOLET SPECTRUM (ETHANOL)

N-METHYLLYCODINE


N.M.R. SPECTRUM ($CDCl_3$)
TMS
β-OBSCURINE (XXIII)
2.21
3.43
7.46
9.17
TMS


N.M.R. SPECTRUM ($CDCl_3$)
α-OBSCURINE (XXIII)
1.99
7.57
9.14
TMS

THE STRUCTURE OF LYCODOLINE (ALKALOID L-8).

Following the elucidation of the structure of the obscurines and lycodine, we turned out attention to another of the minor alkaloids of Lycopodium annotinum L. which we had isolated. This was the alkaloid which Manske and Marion had designated alkaloid L-8 when it was first isolated from L. annotinum Linn. in 1943 (18). This substance was particularly interesting since it is one of the more widespread of the Lycopodium alkaloids, having also been isolated from L. annotinum var. acrifolium (53) (the substance as isolated from this species was originally designated alkaloid L-30, but it was later shown (54) that in fact L-30 is identical to L-8), L. selago (55), L. fawcettii (56), and L. clavatum (28). More recently it has also been isolated from L. lucidulum (28) and L. prostratum (57). We have chosen the name lycodoline for alkaloid L-8.

Lycodoline was isolated from the crude "annotinine free" alkaloids of L. annotinum by elution chromatography as described in the Experimental. The presence of a sharp characteristic band at 960 cm^{-1} in the infrared spectrum facilitated the isolation of lycodoline. Lycodoline elutes and cocrystallizes with the residual annotinine present in the "annotinine free" crude alkaloid. As previously described the majority of the annotinine is removed by crystallization of the crude alkaloid from 95% ethanol. Lycodoline

may be separated from annotinine by treatment of the mixture with aqueous ethanolic potassium hydroxide. Annotinine (I) is transformed to the corresponding amphoteric amino acid by opening of the lactone and remains in the aqueous solution while the lycodoline is extracted into chloroform. The lycodoline used in this investigation was identical to an authentic sample of alkaloid L-8 kindly supplied by Dr. R.H.F. Manske.

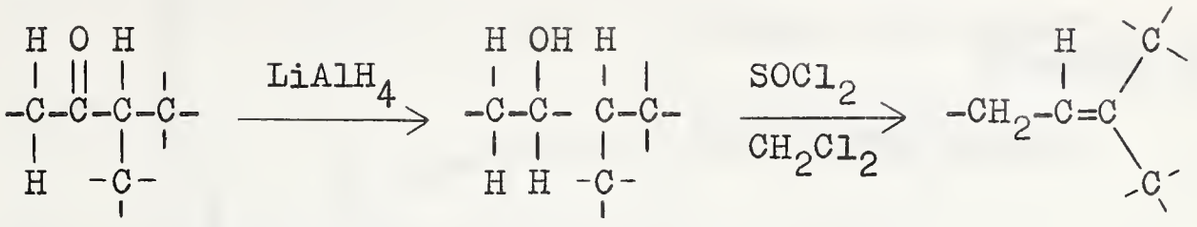
The analytical results of earlier workers (18,53,54,55, 56), indicated a molecular formula $C_{16}H_{25}O_2N$ for the alkaloid. Perry and MacLean had shown (58) on the basis of the infrared spectrum that the oxygen atoms are present as a carbonyl group (1710 cm^{-1}) and a hydroxyl group (3220 cm^{-1}). Burnell (56) showed the absence of an N-methyl group and reported the presence of one active hydrogen (attributable to the hydroxyl) in the molecule. This supported the earlier suggestion (58) that the nitrogen is tertiary.

Our analytical and spectral data confirmed these characteristics and in addition showed the presence of a $>CHCH_3$ group in the molecule (production of 0.68 moles of volatile acid on Kuhn - Roth oxidation in conjunction with a three proton doublet centered at τ = 9.14 in the N.M.R. spectrum). The N.M.R. spectrum showed no signals below τ = 6.5 and this fact, coupled with the failure of lycodoline to take up hydrogen on attempted catalytic hydrogenation and the absence of absorption in the infrared in the 1600 - 1680 cm^{-1} region, indicated the absence of olefinic linkages. This data suggested that the molecule is tetracyclic.

The next step in the investigation of the structure was the definition of the environment about the oxygen functions.

The carbonyl group appeared to be a part of a six (or larger) membered ring as indicated by the position of the carbonyl stretching vibration in the infrared spectrum (CCl_4, 1703 cm^{-1}) (35). There appeared to be a methylene group α to the carbonyl as indicated by a band at 1410 cm^{-1} in the infrared spectrum (59). Reduction of the carbonyl with $LiAlH_4$ gave dihydrolycodoline ($C_{16}H_{27}O_2N$, M.P. 186-187°) which showed a new band in the infrared spectrum at 3625 cm^{-1} (-OH). Dehydration of dihydrolycodoline with $SOCl_2$ in CH_2Cl_2 (lycodoline itself is stable to this reagent) gave anhydrodihydrolycodoline ($C_{16}H_{25}ON$) characterized as its hydrobromide (M.P. 293-295°). The infrared spectrum of the salt showed a weak band at 1665 cm^{-1} ($>C=C<$) while the N.M.R. spectrum of the free base showed a one proton signal as an unresolved multiplet at τ = 4.56. The evidence given by the N.M.R. spectrum shows that the dehydration does not involve one of the hydrogen atoms of the α-methylene group because, if this were the case, there would be two low field protons in the spectrum. Thus the dehydration must take place by means of a hydrogen atom from the alternate α carbon. Since the N.M.R. spectrum shows the presence of only one olefinic hydrogen, and this hydrogen must be attached to the carbon which was originally in the form of a carbonyl group, it follows that one of the α-carbon atoms in lycodoline carries only one hydrogen atom. The following partial

structures incorporate the information presented above.



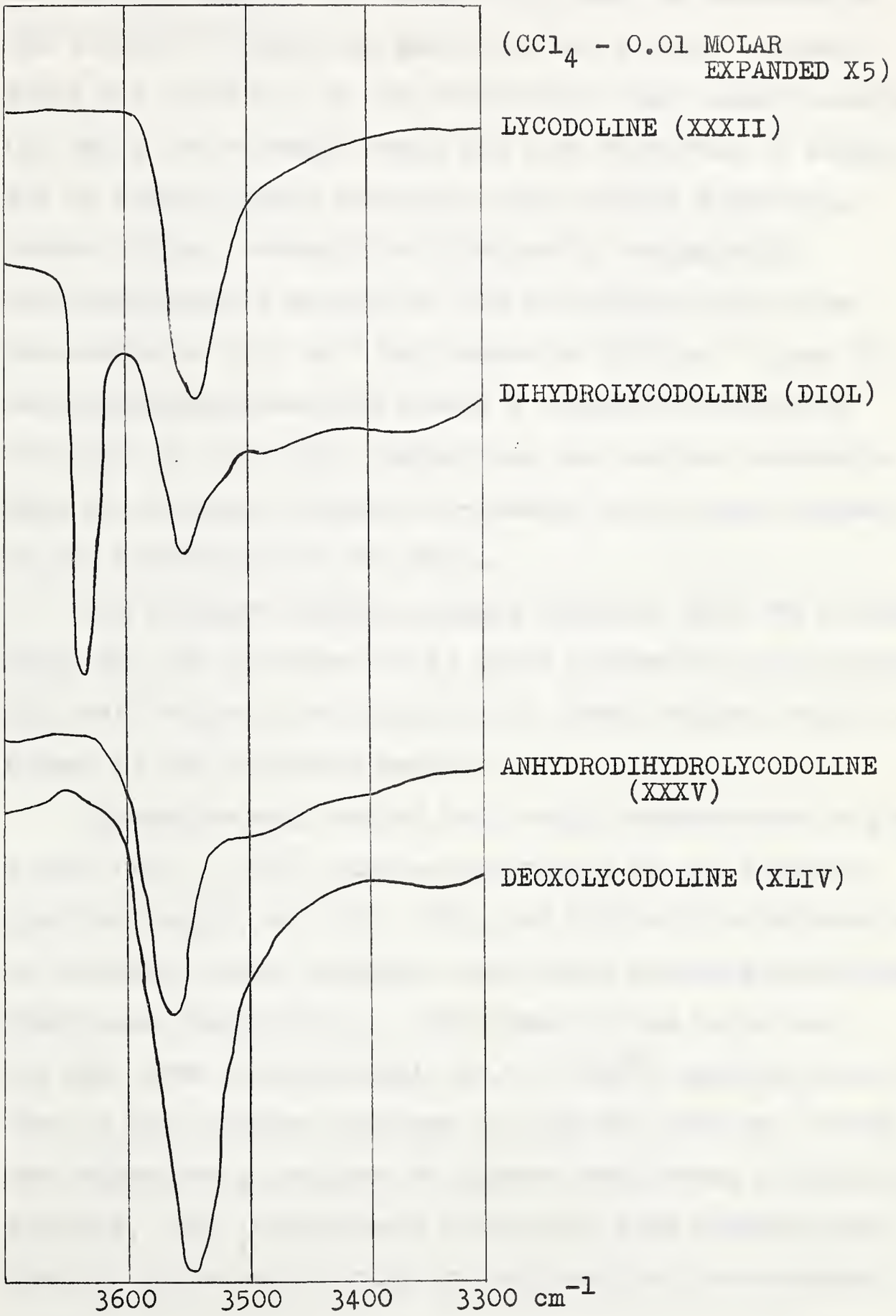
Attempts to catalytically reduce the olefin were unsuccessful.

The environment of the carbonyl group, coupled with the apparently hindered nature of the olefin in the anhydro-compound, is reminiscent of lycopodine chemistry, in which an analogous situation is encountered (14).

Lycodoline proved to be resistant to acetylation under various conditions, resistant to oxidation (CrO_3-pyridine; CrO_3-acetic acid), and, as mentioned before, showed no absorption in the N.M.R. spectrum below τ = 6.5. The chemical and physical data indicates that the hydroxyl group in lycodoline is tertiary.

The position of the -OH stretching vibration in the infrared spectra of lycodoline and its derivatives was especially informative. The infrared spectrum of lycodo-line, measured in dilute carbon tetrachloride, showed a concentration independent band at 3545 cm^{-1} (see page 59). It is known (60) that intramolecular hydrogen-bonding causes a concentration independent shift of the -OH stretching band from the non-bonded 3615-3635 cm^{-1} region to lower frequency. We attribute the shift observed in lycodoline to intramolecu-lar hydrogen-bonding between the hydroxyl group and the

HYDROGEN-BONDING LYCODOLINE AND DERIVATIVES.

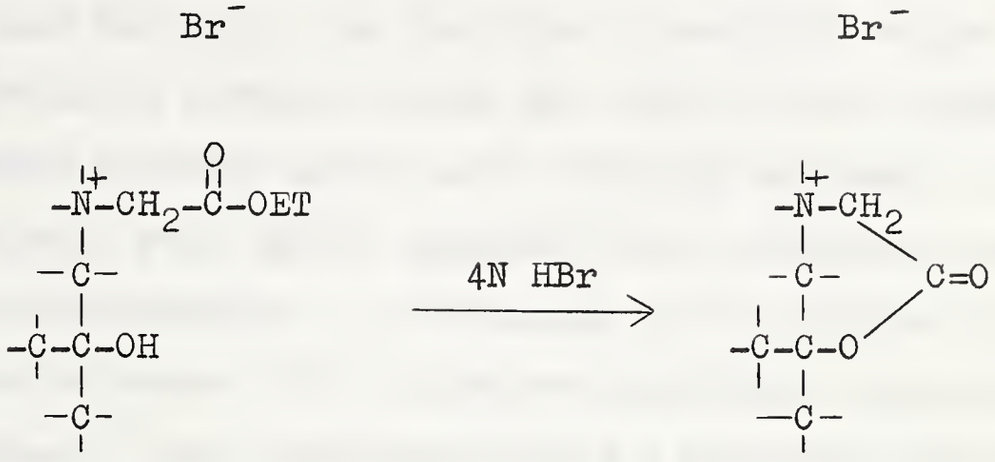


tertiary nitrogen. The possibility that the association was between the hydroxyl group and the ketonic carbonyl group was ruled out by the observation that deoxolycodoline (in which the carbonyl group had been converted to methylene by Wolff-Kishner reduction) also showed a hydrogen-bonded hydroxyl absorption (3545 cm^{-1}, see page 59). Dihydrolycodoline showed two -OH stretching vibrations, non-bonded at 3625 cm^{-1} and bonded at 3550 cm^{-1} (page 59). Anhydrodihydrolycodoline showed a single OH stretching vibration at 3565 cm^{-1}, supporting our earlier assumption that the original hydroxyl (H-bonded) is the one retained in the dehydration of the diol.

The hydrogen bonding studies indicate that the hydroxyl group and the nitrogen are in close proximity to one another. The fact that this relationship is indeed vicinal was confirmed in the following manner:

Lycodoline was treated with ethyl bromoacetate to give a salt (M.P. $>330^{\circ}$) showing absorption in the infrared spectrum (nujol) at 3240, 1753, and 1717 cm^{-1} attributable to hydroxyl, ester carbonyl, and ketone carbonyl stretching vibrations respectively. Hydrolysis of the ester-salt (4N HBr) gave a lactone-salt (M.P. $>330^{\circ}$) showing absorption in the infrared spectrum at 1756 and 1705 cm^{-1} which are respectively assigned to lactone and ketone carbonyl absorption. The lactone-salt would also form spontaneously from the ester-salt during crystallization from methanol.

These compounds are illustrated by the following partial structures.

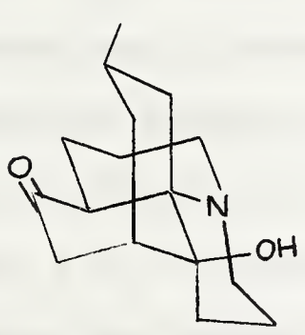


Ester-Salt Lactone-Salt

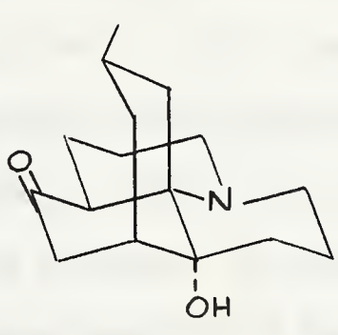
The ease of formation of the lactone-salt from the ester-salt coupled with the fact that the lactone carbonyl absorbs at essentially the same place in the infrared as does the ester carbonyl, strongly suggests that the lactone is six membered. If the lactone is indeed six membered, then there must be a single carbon between the nitrogen atom and the carbon which carries the hydroxyl group.

To support the assignment of the ester-salt and lactone-salt carbonyl absorption bands in the infrared spectra, analogous compounds were prepared from deoxolycodoline. The ester-salt of deoxolycodoline (M.P. 212°) showed absorption in the infrared spectrum (nujol) at 3248 cm^{-1} (-OH) and at 1747 cm^{-1} (ester carbonyl). The infrared spectrum (nujol) of the lactone-salt of deoxolycodoline (M.P. 327°) was free of hydroxyl absorption but showed the lactone carbonyl stretching vibration at 1754 cm^{-1}. These observations support the interpretations made in the case of the ester and lactone-salts of lycodoline.

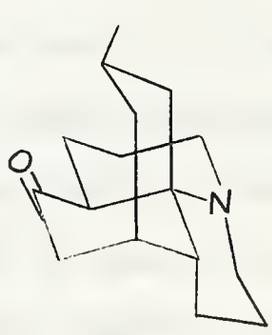
The apparent similarity between the environment of the carbonyl group in lycodoline and in lycopodine was mentioned earlier. The fact that lycodoline occurs with lycopodine in several plants and that it also contains a secondary C-methyl group and a tertiary nitrogen in a tetracyclic ring system suggested that lycodoline might be a hydroxylycopodine. A comparison of the optical rotatory dispersion curves (61) of the two compounds strengthened this view. Thus lycopodine showed a positive Cotton curve $[\alpha]^{MeOH}_{307\ m\mu}$ + 2300°, $[\alpha]^{MeOH}_{265\ m\mu}$ -6300° as did lycodoline $[\alpha]^{MeOH}_{308\ m\mu}$ + 2660°, $[\alpha]^{MeOH}_{265\ m\mu}$ - 6900°. Assuming the two molecules have the same absolute configuration, and taking into account the vicinal relationship of the nitrogen atom and the hydroxyl group, structure XXXII becomes a possibility for lycodoline.



XXXII



XXXIII

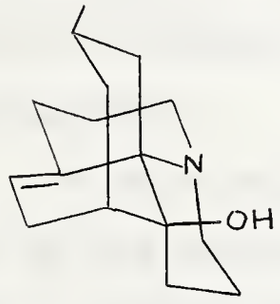


XXXIV

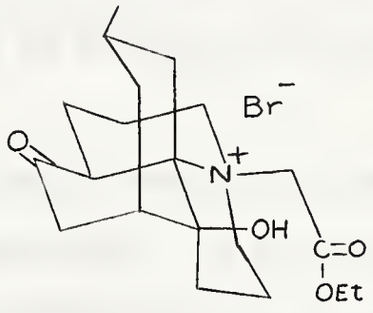
However, according to the Octant Rule (61), substituents at C-4 of the carbonyl containing ring have no effect upon the dispersion curve and thus structure XXXIII is an equally

probable representation of lycodoline. The structure of lycopodine (XXXIV) is included for comparison.

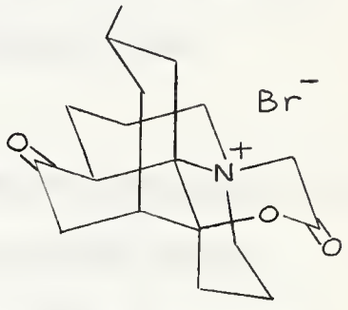
If structure XXXII is correct for lycodoline, then anhydrodihydrolycodoline may be represented by XXXV and the ester-salt and lactone-salt by XXXVI and XXXVII respectively.



XXXV



XXXVI



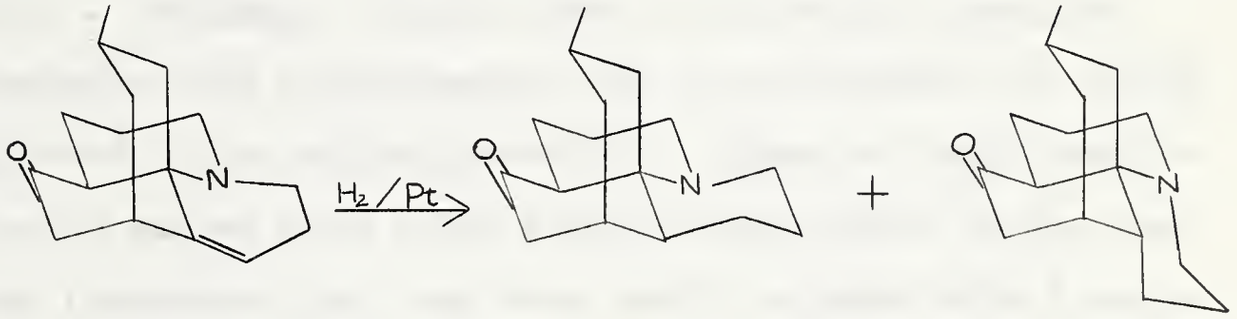
XXXVII

Analogous structures may be written for XXXV, XXXVI, and XXXVII using XXXIII as the structure for lycodoline.

Throughout the early part of the investigation of the structure of this alkaloid attempts were made to dehydrate lycodoline itself. The reagents used in these various attempts included $POCl_3$/pyridine, $SOCl_2$/benzene, $SOCl_2/CH_2Cl_2$, 85% H_3PO_4, H_2SO_4, P_2O_5 in refluxing toluene, and P_2O_5 in refluxing xylene. In general, it was found that the hydroxyl group could not be easily removed. When more rigorous conditions were used, the yield of basic material decreased and some of the infrared spectra of the crude reaction products indicated that the carbonyl group might be in some way effected. Concurrently, it was found,

however, that deoxolycodoline (the Wolff-Kishner reduction product of lycodoline), which could be obtained in 85% yield from lycodoline, could be dehydrated in 90% yield by P_2O_5 in refluxing toluene and the dehydration product isolated as a stable, crystalline hydrochloride. Catalytic reduction of this salt was successful and gave a stable oil which was easily purified by means of the crystalline perchlorate.

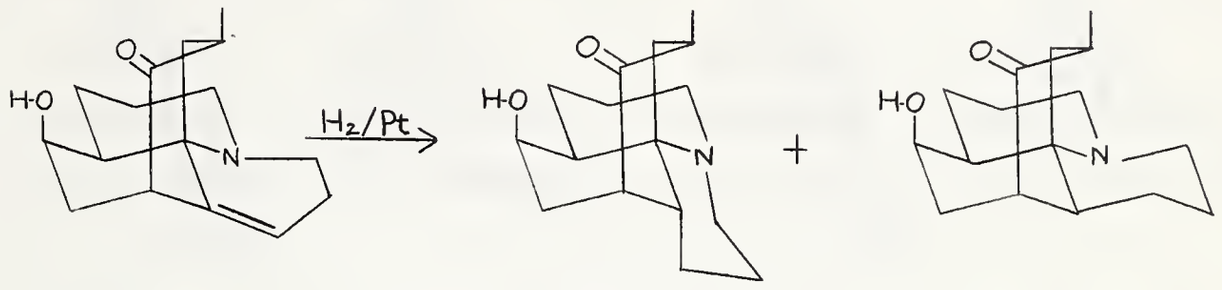
At this point, a decision had to be made concerning the use of the available lycodoline. If it is assumed that either structure XXXII or XXXIII is the correct representation of lycodoline, then direct relationship with lycopodine (XXXIV) appeared to be the most logical approach, requiring only a dehydration to XXXVIII and a hydrogenation to give some yield of lycopodine according to the following scheme.



XXXVIII XXXIX XXXIV

The yield of lycopodine would be dependent upon the extent to which XXXVIII would approach the catalyst from the side which carried the methyl bearing ring. Anet (39) has provided an analogy for the reaction by the conversion

of acrifoline (XLI) (62,63) to annofoline XLII by catalytic hydrogenation. The crude product of the hydrogenation in



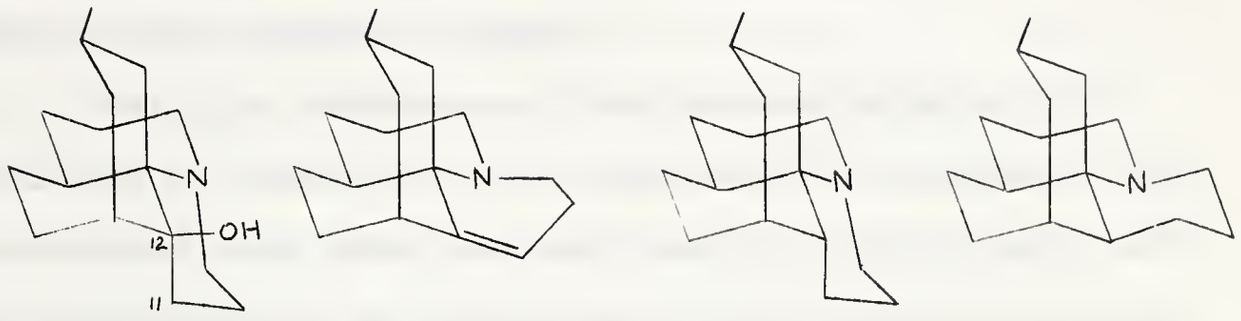
XLI XLII XLIII

this case is made up of approximately 10% annofoline (XLII) and 90% dihydroacrifoline (XLIII).

The only apparent barrier to using the dehydration - hydrogenation scheme in the case of lycodoline was the difficulty encountered in the dehydration. Conditions under which deoxolycodoline underwent successful dehydration (P_2O_5 - refluxing toluene) gave at least 50% unreacted lycodoline and approximately 10% of an unstable oil which appeared to be anhydrolycodoline. Since we could realistically expect only a 10% yield of lycopodine in the next step (hydrogenation) and this would be mixed with a major component (XXXIX) of presumably similar characteristics, the route was abandoned because of the limited supply of alkaloid on hand at that time.

Again, if structure XXXII or XXXIII is correct for lycodoline, then deoxolycodoline would be XLIV (or the

C-12 epimer), the product of dehydration would be XLV, and



XLIV XLV XLVI XLVII

the products of catalytic reduction would be XLVI and XLVII with XLVII predominating. As mentioned before, XLIV, XLV and XLVII could apparently be obtained from lycodoline in reasonable yield so we chose to attempt a direct correlation of lycodoline with a degradation product of acrifoline (XLI) and thus establish unambigously the carbon skeleton of lycodoline.

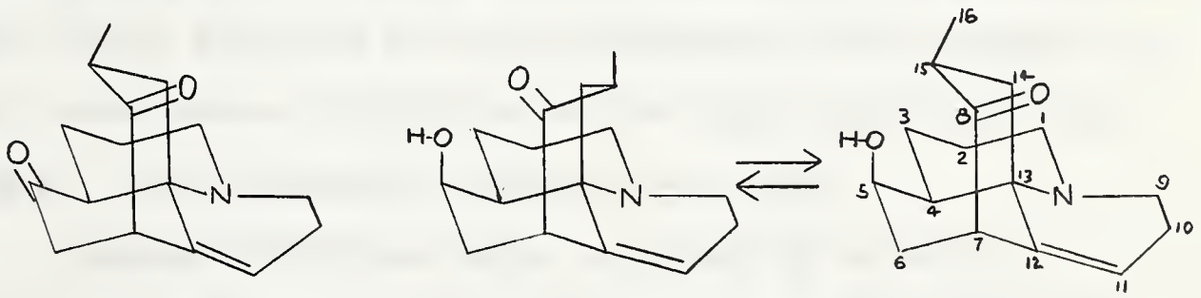
Deoxolycodoline ($C_{16}H_{27}ON$, M.P. 123°) prepared from lycodoline by the Barton modification (64) of the Wolff-Kishner reaction was free of carbonyl absorption in the infrared spectrum and showed the previously mentioned hydrogen bonded hydroxyl stretching vibration at 3545 cm^{-1}.

Treatment of deoxolycodoline with P_2O_5 in refluxing toluene gave anhydrodeoxolycodoline (XLV) conveniently isolated as the crystalline hydrochloride by chloroform extraction of a solution of XLV in dilute HCl. The infrared spectrum of the free base was free of absorption due to hydroxyl and carbonyl stretching vibrations.

The N.M.R. spectrum of XLV showed a one proton signal as a poorly resolved triplet at τ = 4.64 indicative of a tri-substituted olefinic linkage.

Catalytic reduction of the hydrochloride of XLV followed by recovery of the free base and treatment with perchloric acid gave the perchlorate of XLVII (M.P. 241°, $C_{16}H_{27}N$) showing no absorption in the infrared spectrum (nujol) between 1500 and 2800 cm^{-1}. The infrared spectrum of the free base (CCl_4) shows a series of well developed bands in the 2750 - 2850 cm^{-1} region, the significance of which will be discussed later.

Oppenauer oxidation (65) of acrifoline gave a diketone, characterized as its hydrobromide ($C_{16}H_{21}O_2N.HBr$, M.P. 309-310°, sealed tube) which showed carbonyl absorption in the infrared (nujol) at 1728 and 1714 cm^{-1}. The diketone is assigned the stereochemistry indicated by XLVIII since it has been shown by Anet (39) that under equilibrating conditions the boat form of acrifoline (XLI) is in equilibrium



XLVIII XLI XLIa

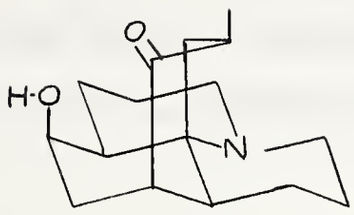
with the chair form (XLIa). Thus it would appear that the sum of the nonbonded interactions which are operative when

ring D is in the boat conformation is to some extent balanced by the hydroxyl - C-15 interaction operative when ring D is in the chair conformation. The transformation of C-5 from a tetrahedral to a trigonal arrangement (oxidation of the hydroxyl to the ketone) removes the hydroxyl - C-15 interaction and allows the methyl bearing ring to remain in the chair conformation. The methyl group at C-15 can epimerize under the basic conditions of the Oppenauer reaction leading to the diketone XLVIII.

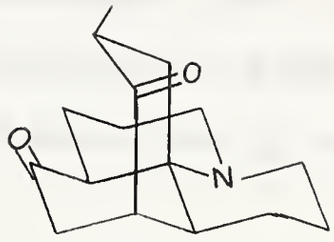
Reduction of the diketone XLVIII using the Barton modification of the Wolff-Kishner method gave anhydrodeoxolycodoline (XLV) isolated as the hemi-hydrate hydrochloride and identical to the hemi-hydrate hydrochloride of an authentic sample prepared from lycodoline (comparison of infrared spectra ($CHCl_3$), M.P. undepressed M.M.P. and optical rotation).

Catalytic reduction of XLV obtained from acrifoline gave XLVII isolated as its perchlorate and identical to the perchlorate of XLVII obtained from lycodoline (M.P., M.M.P., superimposable infrared spectrum).

Compound XLVII was also obtained by catalytic reduction of acrifoline to dihydroacrifoline (XLIII) which in turn was oxidized (Oppenauer) to the previously reported (62) diketone XLIX. Wolff-Kishner reduction of XLIX gave XLVII as expected.



XLIII



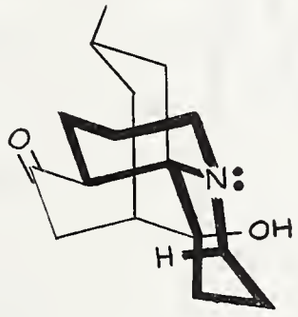
XLIX

This series of reactions establishes the carbon skeleton of lycodoline and provides firm evidence in support of the placement of the hydroxyl group at C-12. However the series does not directly distinguish between structures XXXII and XXXIII since dehydration of the two possible deoxo compounds would result in the same anhydro-deoxo compound XLV.

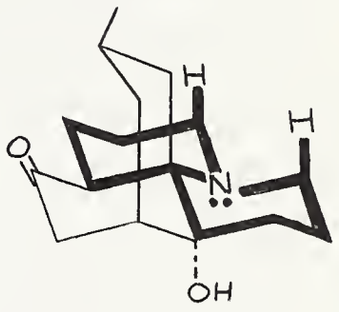
Since the Wolff-Kishner reduction of XLVIII and XLIX could conceivably result in the epimerization of the methyl group (66) the assignment of this group to the equatorial conformation with ring D in the chair form is not completely rigorous.

The conformation about the nitrogen atom in lycodoline and thence the choice between structure XXXII and XXXIII rests upon infrared spectral evidence. Bohlman (67,68) has shown that a series of absorption bands between 2750 and 2850 cm^{-1} are prominent in the infrared spectrum of a quinolizidine system when at least two α carbon-hydrogen bonds are orien-

tated transdiaxially to the electron pair on the nitrogen. Structure XXXII contains one carbon-hydrogen bond in such a position while structure XXXIII contains two such bonds. In each case the pertinent system is described by heavy lines.



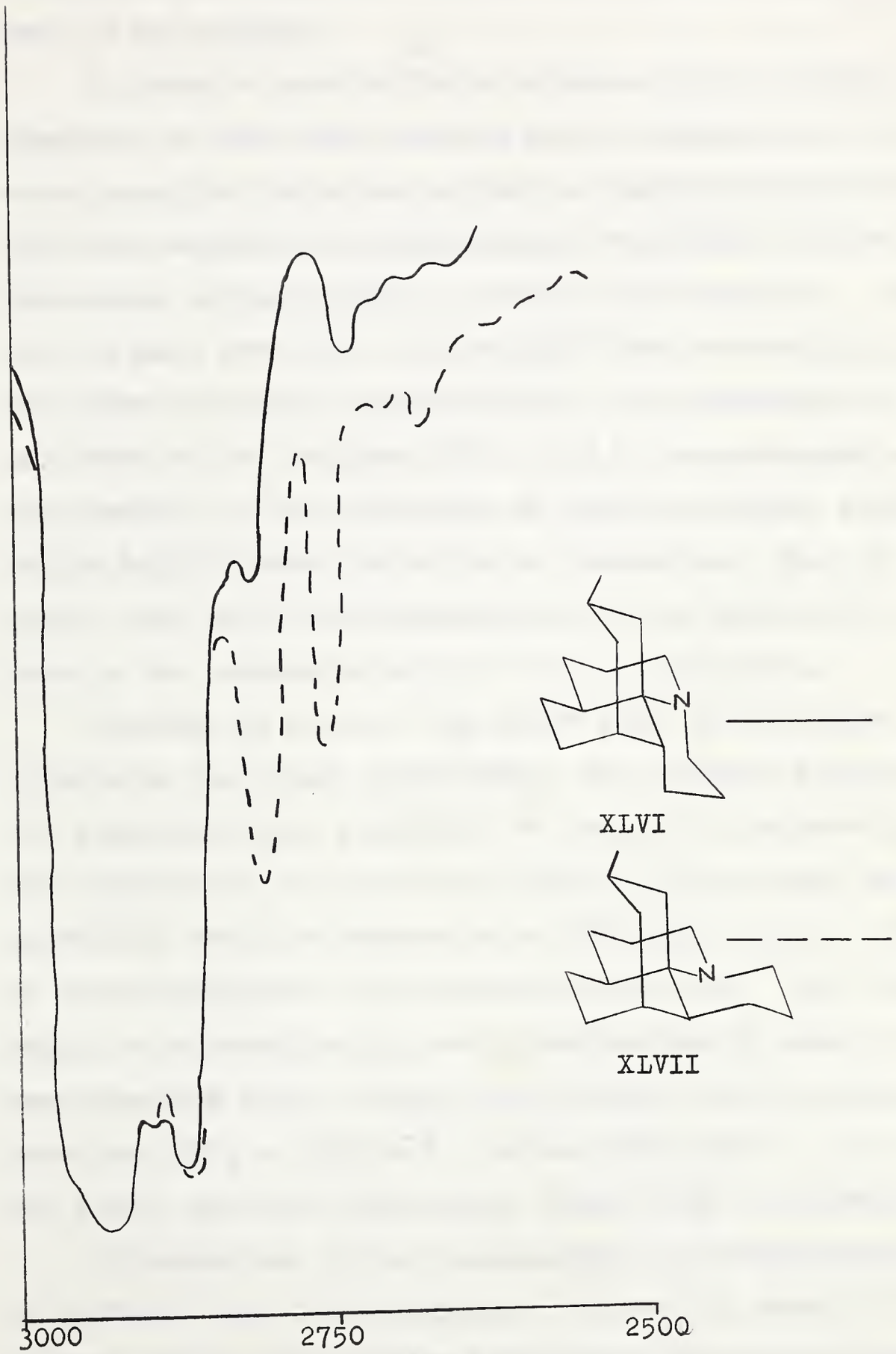
XXXII



XXXIII

Dihydroanhydrodeoxolycodoline (XLVII) has well developed bands in the 2750 - 2850 cm^{-1} region of the infrared spectrum (CCl_4) while the infrared spectra of lycodoline, lycopodine (XXXIV), and deoxolycopodine (XLVI) (the Wolff-Kishner reduction product of lycopodine) are relatively free of absorption in this region. For purposes of illustration, the pertinent regions of the infrared spectra (CCl_4) of the isomers XLVI and XLVII are shown on page 71.

It may be concluded that a cis quinolizidine system is incorporated in the structure of lycodoline (just as in lycopodine and deoxolycopodine) and that a trans quinolizidine system is present in XLVII. The conformation about the nitrogen in XLVII must then be reversed to that in


3000
2750
2500
N
XLVI
N
XLVII

lycodoline and thus the correct representation of lycodoline must be as in XXXII.

In order to gain definite evidence for the stereochemistry of the methyl bearing ring in lycodoline, the crude catalytic reduction product of anhydrodeoxolycodoline (XLV) was carefully chromatographed over basic alumina using thin-layer chromatography to monitor the fractions. In this way the main reduction product XLVII was successfully separated from the minor component XLVI. The methiodide of XLVI was shown to be identical (M.P., M.M.P., superimposable infrared spectra) to the methiodide of deoxolycopodine, prepared by the Wolff-Kishner reduction of lycopodine. This of course proves that the stereochemistry at C-15 in lycodoline is the same as the stereochemistry at C-15 in lycopodine.

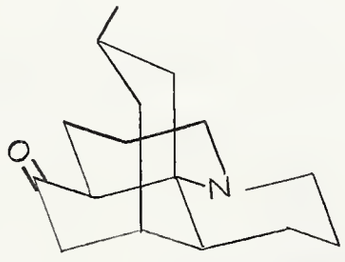
Although we felt at this point that the structure of lycodoline was firmly established, when further supplies of the alkaloid became available, we decided to reinvestigate the dehydration of lycodoline itself. It was found that lycodoline could be dehydrated in 90% yield using a solution of phenylphosphonic dichloride-pyridine (1:1). The product which is an unstable oil, was characterized by means of its mass spectrum (M.W. = 245, $C_{16}H_{25}O_2N-H_2O$ = 245), infrared spectrum (CCl_4 - 1698 cm^{-1} - ketone; 1650 cm^{-1} - $>C=C<$), and N.M.R. spectrum (one proton signal at τ = 4.44-multiplet).

Hydrogenation of the perchlorate of anhydrolycodoline in methanol over Adam's catalyst followed by careful elution chromatography over alumina again using thin-layer chromato-

graphy (alumina, benzene: ethyl acetate 1:1) as fraction monitor gave lycopodine (approximately 15%), identical to an authentic sample (M.P., M.M.P., superimposable infrared spectra and optical rotatory dispersion curves).

This result confirms all the previous evidence for structure XXXII for lycodoline and in particular, the location of the carbonyl group.

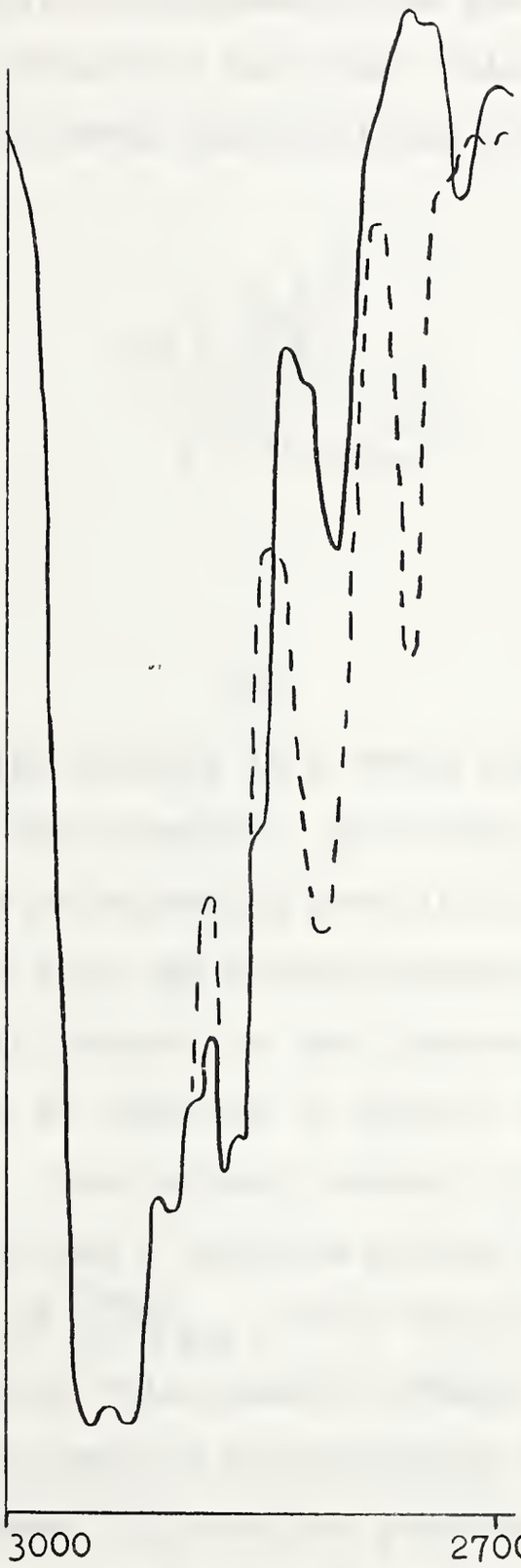
The main product of the catalytic reduction ($C_{16}H_{25}ON$, M.P. 89-90°) showed well developed bands in the infrared through the 2750 - 2850 cm^{-1} region as well as carbonyl absorption at 1698 cm^{-1}. This compound is assigned structure XXXIX by analogy with other hydrogenation reactions and by

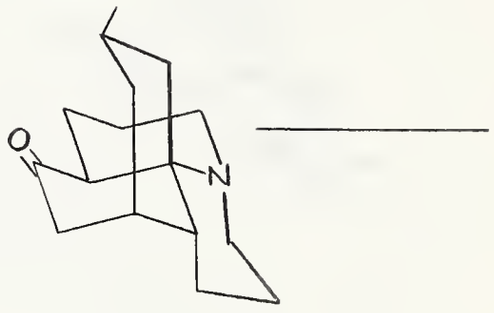


XXXIX

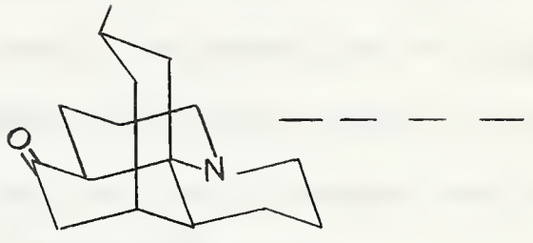
the presence of the typical trans quinolizidine carbon-hydrogen stretching absorption in the infrared spectrum. The pertinent portions of the infrared spectra of XXXIX and lycopodine are provided on page 74 and these constitute further support for the assignment of the conformation about the nitrogen in lycodoline.

Acrifoline (XLI) also possesses fairly well developed



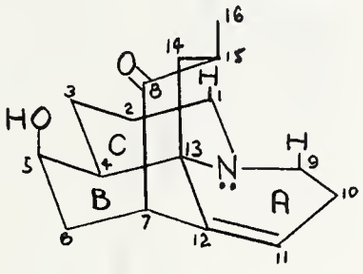


XXXIV

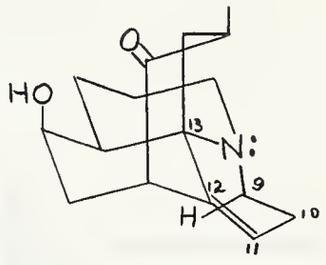


XXXIX

absorption bands in the 2750 - 2850 cm^{-1} region of the infrared spectrum (see page 76) and the existence of this absorption may be interpreted to mean that acrifoline exists predominantly in the ring A conformation as depicted by structure XLI since this conformation contains an unsaturated trans quinolizidine system with two α -carbon-hydrogen



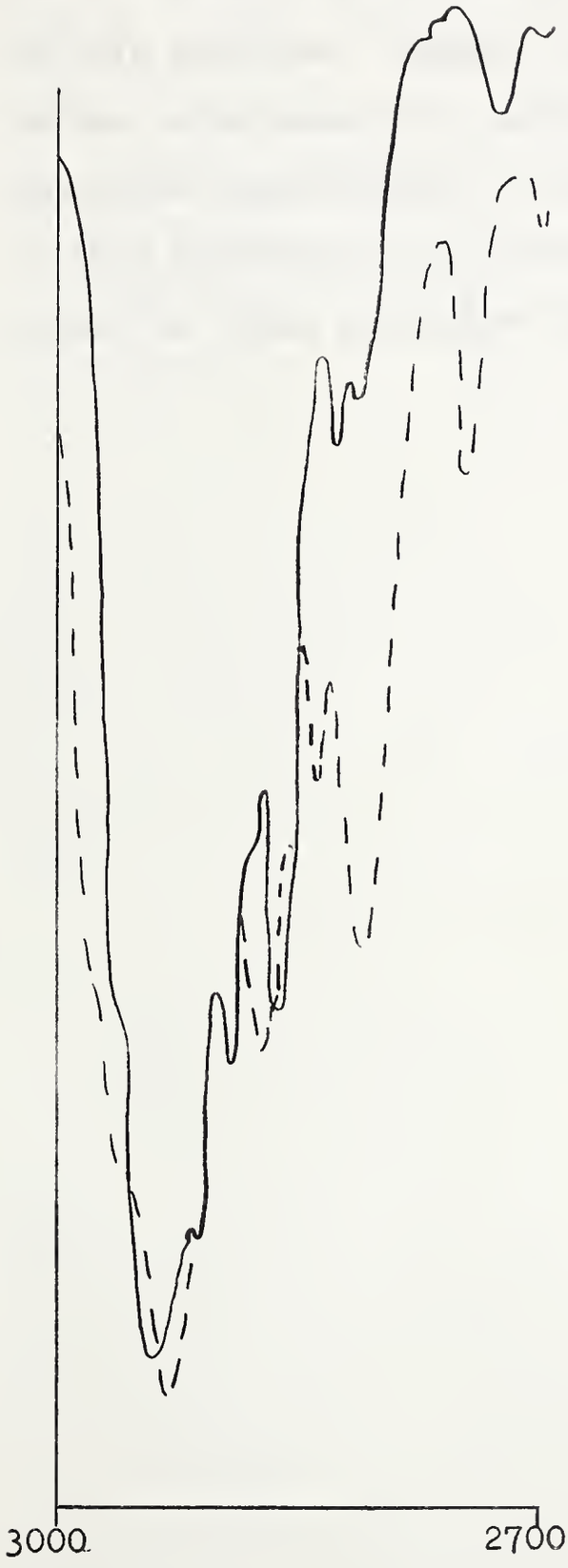
XLI

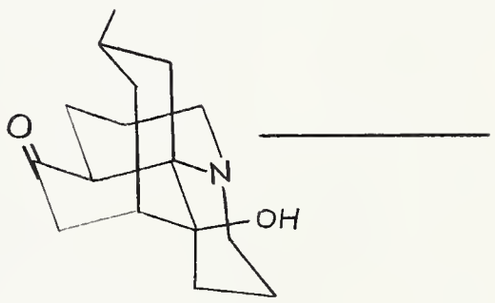


XLIb

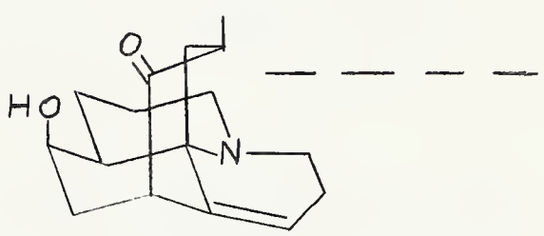
bonds located in a trans diaxial sense to the electron pair on the nitrogen. The other possible conformation XLIb (with the nitrogen-C-9 bond in the axial conformation to ring C) has only one carbon-hydrogen bond trans diaxially situated with respect to the electron pair on the nitrogen and would not be expected to exhibit the "Bohlman bands".

The optical rotatory dispersion curve of compound XXXIX exhibits a negative Cotton effect $\left[\phi\right]^{MeOH}_{306\ m\mu}$ - 2300° $\left[\phi\right]^{MeOH}_{265\ m\mu}$ + 2800° which is anomalous in view of the established relationship between lycodoline and lycopodine. Since the absolute configuration of lycopodine has been thoroughly tested (14) and the structure of XXXIX was arrived at unambiguously, the cause of the anomoly is not readily apparent.





LYCODOLINE



ACRIFOLINE

Since optical rotatory dispersion curves have been unavailable for some time due to instrument failure, we have not been able to carry out further investigations on this problem. However, the nitrogen atom electron pair-ketone relationship in lycopodine is different from the analogous relationship in compound XXXIX. The effect of this difference in relationship between the functional groups in these molecules remains untested.

EXPERIMENTAL LYCODOLINE

Lycodoline was isolated from Lycopodium annotinum Linn by elution chromatography of that portion of "annotinine free" crude alkaloid which is eluted from the initial chromatogram with ether. Elution with ether, ether:methylene chloride, and methylene chloride gave fractions which contained lycodoline as detected by a sharp characteristic band in the infrared spectrum at 960 cm^{-1}. Suitable combination of fractions and crystallization from acetone gave lycodoline (M.P. 180-181°). Sublimation (167°/0.5 mm) of constant melting material gave the analytical sample. Infrared (CCl_4) 3545 cm^{-1} (-OH), 1703 cm^{-1} ($\rangle C=O$). (FOUND: C, 72.58; H, 9.62; N, 5.49; C-CH_3, 3.85%. CALCULATED FOR $C_{16}H_{25}O_2N$: C, 72.95; H, 9.57; H, 9.57; N, 5.32; C-CH_3, 5.69%).

1. $LiAlH_4$ REDUCTION OF LYCODOLINE:

Lycodoline (148 mg) in ether (100 ml) was added to a mixture of $LiAlH_4$ (180 mg) in ether (75 ml) and the solution was refluxed for 18 hours. The excess $LiAlH_4$ was destroyed with wet ether and the resulting solution was acidified with dilute hydrochloric acid and the two phase system shaken thoroughly. The aqueous acid solution was made basic with NH_4OH and extracted with $CHCl_3$. Evaporation of the dried ($MgSO_4$) $CHCl_3$ extract gave dihydrolycodoline (100 mg) which upon recrystallization from

acetone yielded the analytical sample M.P. 186 - 187°. Infrared (CCl_4, 0.01 Molar, 0.005 Molar) 3629, 3550 cm^{-1} (concentration independent).
(FOUND: C, 72.16, 71.99; H, 10.14, 10.11%. CALCULATED FOR $C_{16}H_{27}O_2N$: C, 72.41; H, 10.26%).

2. THE DEHYDRATION OF DIHYDROLYCODOLINE:

A solution of dihydrolycodoline (91 mg), CH_2Cl_2 (20 ml), and $SOCl_2$ (1.5 ml) was allowed to stand at room temperature for two and one half hours. The solvent and excess $SOCl_2$ was removed at reduced pressure (water pump) and the oily residue was dissolved in dilute hydrochloric acid. The aqueous acid solution was washed with ether and basified with concentrated NH_4OH. The basic solution was extracted with $CHCl_3$ which gave a pale yellow oil (68 mg) upon drying ($MgSO_4$) and evaporation. Chromatography over basic alumina (2 g) gave, by elution with ether, a colorless oil (62 mg) which was transformed to the hydrobromide in acetone solution. Recrystallization from acetone gave the analytical sample M.P. 293-295°. (FOUND: C, 58.62; H, 8.11%. CALCULATED FOR $C_{16}H_{25}ON.HBr$: C, 58.55; H, 7.99%). Distillation (130°/0.5 mm) of a sample of free base recovered from the hydrobromide salt showed bands in the infrared (CCl_4, 0.05 molar, 0.01 molar) at 3565 cm^{-1} (concentration independent) 1650 (V. Weak). The N.M.R. spectrum of the free base showed a one proton signal as a poorly resolved multiplet at $\tau = 4.56$ ($-\overset{H}{C}=\overset{H}{C}-$) and a three proton signal

at τ = 9.08 as poorly defined doublet. Addition of HCl to the sample gave a well resolved doublet (J = 5 c.p.s.) centered at τ = 9.03.

A portion of anhydrodihydrolycodoline (17 mg) was dissolved in methanol (50 ml) and shaken with Adams catalyst (40 mg) at a hydrogen pressure of 40 p.s.i. The catalyst was filtered and the filtrate evaporated to a crystalline residue which upon recrystallization from acetone proved to be unreacted anhydrodihydrolycodoline.

3. WOLFF-KISHNER REDUCTION OF LYCODOLINE:

Hydrazine which had been refluxed over NaOH pellets for 3 hours was distilled into a solution of diethylene glycol (25 ml) and sodium (0.5 g). The resulting solution was distilled until the solution temperature reached 190°. The solution was allowed to cool to 50° and lycodoline (200 mg) was added. This solution was refluxed at a solution temperature of 200-208° for 17½ hours. The cooled reaction mixture was diluted with water, acidified with 6N HCl, and continuously extracted with ether for 18 hours, the layers were separated and the aqueous layer was made basic with concentrated NH_4OH and extracted with $CHCl_3$. The dried $CHCl_3$ extract was evaporated to a crystalline residue (292 mg contaminated with a small amount of diethylene glycol). The deoxolycodoline was separated from the contaminant by recrystallization from acetone, and perchlorate formation of the acetone mother

liquors. In this manner, deoxolycodoline was obtained in 80-90% yield from lycodoline.

The analytical sample was obtained by recrystallization from acetone to constant melting point (122.5-123.5°). Infrared (CCl_4, 0.05, 0.01 M) 3545 cm^{-1} (concentration independent). (FOUND: C, 76.51, 76.84; H, 10.65, 10.74; O, 6.82; N, 5.57. $C_{16}H_{27}ON$ requires: C, 77.05; H, 10.91; O, 6.42; N, 5.62%).

The crude perchlorate obtained from the acetone mother liquors was recrystallized from acetone-ethyl acetate to constant melting point (273-275°). (FOUND: C, 55.13, 54.95; H, 8.28, 8.07; N, 4.14. $C_{16}H_{27}ON.HClO_4$ requires: C, 54.95; H, 8.07; N, 4.01%).

4. PREPARATION OF THE ESTER-SALT XXXVI:

A solution of lycodoline (45 mg), ethyl bromacetate (1.5 ml), and benzene (4 ml) was refluxed for two hours. Filtration of the cooled reaction solution gave XXXVI (40 mg) which after recrystallization from methanol-ether did not melt below 340°. Infrared (nujol) 3240 cm^{-1} (-OH), 1753 cm^{-1} (ester), 1717 cm^{-1} (ketone). (FOUND: C, 55.51; H, 7.46; $C_{20}H_{32}O_4NBr$ requires: C, 55.81; H, 7.50%).

5. PREPARATION OF THE LACTONE-SALT XXXVII:

The ester-salt XXXVI (140 mg) was refluxed in 4.4N HBr for 24 hours. Evaporation of the solvent left an amphorous yellow solid as residue, which was triturated with methanol-

acetone (5:2). The soluble portion (obtained by filtration and evaporation of the filtrate, was recrystallized from methanol to give XXXVII (M.P. $>340^{\circ}$), infrared spectrum (nujol) 1757 cm^{-1} (lactone), 1710 cm^{-1} (ketone). (FOUND: C, 55.85; H, 6.64; Br, 21.50; $C_{18}H_{26}O_3NBr$ requires: C, 56.26; H, 6.82; Br, 20.80%). The ester-salt and lactone-salt both required addition of V_2O_5 to obtain complete combustion of the sample for the analyses.

6. PREPARATION OF THE ESTER-SALT OF DEOXOLYCODOLINE:

Treatment of deoxolycodoline (25 mg) with ethyl bromoacetate in the same way as described in part 4 gave the corresponding ester-salt of deoxolycodoline, M.P. 212-213°. (FOUND: C, 58.74; H, 8.53; Br, 19.78. $C_{20}H_{34}O_3NBr$ requires: C, 57.69; H, 8.23; Br, 19.78%). Infrared spectrum (nujol) 3248 cm^{-1} (-OH), 1748 cm^{-1} (ester carbonyl).

7. PREPARATION OF THE LACTONE-SALT OF DEOXOLYCODOLINE:

A solution of the ester-salt of deoxolycodoline (57 mg) in 4.4N HBr was refluxed for 24 hours. Evaporation of the solvent and recrystallization of the amorphous residue from methanol-ether gave the lactone-salt of deoxolycodoline (analogous to the lactone-salt of lycodoline (XXXVII) M.P. 325-326°. (FOUND: C, 57.85; H, 7.35; Br, 20.28. $C_{18}H_{28}O_2NBr$ requires: C, 58.38; H, 7.62; Br, 21.58%). Infrared spectrum (nujol) 1754 cm^{-1} (lactone-carbonyl).

8. ANHYDRODEOXOLYCODOLINE (XLV) FROM DEOXOLYCODOLINE:

A mixture of deoxolycodoline (143 mg), P_2O_5 (1 g) and toluene (100 ml) was refluxed for 3 hours. The excess P_2O_5 was decomposed with ice, the resulting two phase system was separated and the organic layer was washed thoroughly with dilute HCl. The acid solutions were combined, washed with ether, basified with concentrated NH_4OH, and extracted with $CHCl_3$. The dried ($MgSO_4$) $CHCl_3$ extract was evaporated to leave an oily residue (139 mg) the infrared spectrum of which was free of -OH stretching absorption. The crude dehydration product was dissolved in dilute HCl (with difficulty) and the acid solution was thoroughly extracted with $CHCl_3$. Evaporation of the dried $CHCl_3$ extract left a crystalline residue which upon several recrystallizations from acetone-ether gave the analytical sample (M.P. 232-233.5°). Infrared spectrum (nujol) 3485, 3420 cm^{-1} (water of hydration), 2500 cm^{-1} ($>N^{+}H$), 1619 cm^{-1} (water of hydration). (FOUND: C, 69.21, 69.60; H, 9.52, 9.65; Cl, 12.93. $C_{16}H_{25}N.HCl.\frac{1}{2}H_2O$ requires: C, 69.40; H, 9.83; Cl, 12.80%). The N.M.R. spectrum obtained on the free base after purification as the hydrochloride showed a one proton signal at τ = 4.64 as a poorly resolved triplet ($>C = C<^{H}_{CH_2}$); and a 3 proton doublet at τ = 9.16 (J = 5.5 c.ps.) ($CHCH_3$). $(\alpha)_D^{23^\circ} = -26^\circ$ (C = 2.1, ethanol).

9. DIHYDROANHYDRODEOXOLYCODOLINE (XLVII) FROM ANHYDRODEOXOLYCODOLINE (XLV).

The hydrochloride of anhydrodeoxolycodoline (76 mg) was dissolved in methanol (50 ml) and shaken in the presence of Adam's catalyst (25 mg) under a hydrogen pressure of 50 p.s.i. for 3 hours. The catalyst was removed by filtration, the filtrate was evaporated to give a crystalline residue which was dissolved in dilute HCl. The aqueous acid solution was washed with ether, basified with concentrated ammonia, and extracted with chloroform. The dried chloroform extract was evaporated to yield a colorless oil (65 mg) which was dissolved in acetone and treated with perchloric acid. Dilution of the acetone solution with ethyl acetate gave the crystalline perchlorate which, after several recrystallizations from acetone-ethyl acetate, gave the analytical sample (M.P. 240 - 241°). The infrared spectrum (nujol) was free of absorption from 1500 - 2800 cm^{-1}. (FOUND: C, 57.16, 57.18; H, 8.54, 8.51; N, 4.41. $C_{16}H_{27}N.HClO_4$ requires: C, 57.55; H, 8.45; N, 4.20%).

10. DEHYDROACRIFOLINE (XLVIII) FROM ACRIFOLINE (XLI): (OPPENAUER OXIDATION):

A solution of acrifoline (728 mg), cyclohexanone (6 ml), aluminum isopropoxide (800 mg) and toluene (40 ml) was refluxed for 3 hours. The reaction solution was diluted with benzene and extracted with 1N HCl. The acidic extract was washed with ether, basified with concentrated NH_4OH,

and extracted with $CHCl_3$. Evaporation of the dried ($MgSO_4$) $CHCl_3$ extract gave a pale yellow oil (685 mg). Chromatography over basic alumina (12 g) and elution with benzene (500 ml) gave an oily fraction (369 mg) whose infrared spectrum (CCl_4) was free of OH stretch and showed absorption at 1708 and 1712 cm^{-1} (>C = O) and 1665 cm^{-1} (>C = C<) consistent with the expected diketone. Further elution of the column with ether and $CHCl_3$ gave unreacted acrifoline.

Rechromatography over basic alumina (10 g) and elution with benzene (500 ml) gave an oily fraction (230 mg) which was transformed into the hydrobromide in 95% ethanol solution. Dilution of the ethanol solution with ethyl acetate gave the crystalline hydrobromide which upon several recrystallizations from 95% ethanol-ethyl acetate gave the analytical sample (M.P. 309-310° - sealed tube). The infrared spectrum (nujol) of the hydrobromide showed carbonyl absorption at 1728 and 1714 cm^{-1}. (FOUND: C, 56.21, 55.81; H, 6.67, 6.49; N, 3.76. $C_{16}H_{21}O_2N.HBr$ requires: C, 56.56; H, 6.52; N, 4.12%).

11. ANHYDRODEOXOLYCODOLINE (XLV) FROM DEHYDROACRIFOLINE (XLVIII):

Dehydroacrifoline.HBr (210 mg) was added to a solution of sodium, diethyleneglycol, and hydrazine made up exactly as in part 3. The resulting solution was refluxed at a liquid temperature of 186-188° for 13½ hours. The reaction solution was allowed to distil until the solution temper-

ature reached 210°, then refluxing was resumed at a solution temperature of 215-228° for an additional 11 hours. The cooled reaction solution was acidified with dilute HCl and continuously extracted with ether for 27 hours. The aqueous acid solution made basic with concentrated NH_4OH and extracted with $CHCl_3$ which upon drying and evaporation gave a yellow oil which was immediately dissolved in a mixture of dilute HCl and $CHCl_3$ and shaken thoroughly. The aqueous phase was extracted thoroughly with $CHCl_3$, the $CHCl_3$ solutions were combined, dried and evaporated to yield a yellow crystalline residue. Several recrystallizations from acetone-ether gave anhydrodeoxolycodoline hydrochloride hemihydrate identical to the material prepared from lycodoline on the basis of melting point (230-231°), undepressed mixed melting point, superimposable infrared spectra ($CHCl_3$) and optical rotation $(\alpha)_D^{23°}$ = -28° (C = 1.9 Ethanol).

12. DIHYDROACRIFOLINE (XLIII) FROM ACRIFOLINE (XLI):

Acrifoline (500 mg) in methanol (50 ml) was shaken over Adam's catalyst (170 mg) at a hydrogen pressure of 50 p.s.i. for 3 hours. Filtration of the catalyst and evaporation of the methanol gave a pale yellow solid (500 mg) which, upon recrystallization from ether, melted at 168-173°. The infrared spectrum (nujol) showed absorption at 3500 cm^{-1} (-OH), and 1693 cm^{-1} (>C = O). The infrared spectrum (CCl_4) showed absorption at 3610 (-OH),

3470 (bonded -OH), and 1697 ($\rangle C = O$).

13. OXIDATION OF DIHYDROACRIFOLINE TO THE DIKETONE (XLIX):

A solution of dihydroacrifoline (600 mg), cyclohexanone (4 ml), aluminum isopopoxide (500 mg), and toluene (30 ml) was refluxed for 2 hours. The reaction solution was diluted with benzene and extracted with 1N HCl. The acidic extract was washed with ether, basified with concentrated NH_4OH, and extracted with $CHCl_3$. The dried $CHCl_3$ extract was evaporated to yield an oily residue (600 mg) which was chromatographed over neutral alumina (activity I - 15 g). Elution with benzene (200 ml, 200 ml) gave two crystalline fractions (total 143 mg) which, upon recrystallization from pentane gave the analytical sample (M.P. 137-139°) (previously reported 128-129°) (63). The infrared spectrum (CCl_4) was free of hydroxyl stretching absorption and showed carbonyl absorption at 1724, 1712 cm^{-1}. The infrared spectrum (nujol) showed a single carbonyl absorption band at 1714 cm^{-1}. (FOUND: C, 73.02, 73.39; H, 8.53, 8.85; O, 12.90; N, 5.56. $C_{16}H_{23}O_2N$ requires: C, 73.52; H, 8.87; O, 12.25; N, 5.36%).

14. DIHYDROANHYDRODEOXOLYCODOLINE (XLVII) FROM DEHYDRODIHYDROACRIFOLINE (XLIX):

Dehydrodihydroacrifoline (85 mg) was added to a solution of sodium, diethylene glycol and hydrazine made up in exactly the same manner as described in part 3. The reaction solution was refluxed at a solution temperature of 193° for 18 hours.

The cooled reaction solution was acidified with dilute HCl and continuously extracted with ether for 40 hours. The aqueous acid solution was then extracted thoroughly with $CHCl_3$, and the dried $CHCl_3$ extract was evaporated to yield a colorless crystalline residue (32 mg). The free base was recovered and transformed to the perchlorate in acetone solution. Crystallization from acetone-ether gave the perchlorate of dihydroanhydrodeoxolycodoline identical (M.P., M.M.P. and superimposable infrared spectra) to the authentic material prepared from lycodoline.

15. DEOXOLYCOPODINE (XLVI) FROM ANHYDRODEOXOLYCODOLINE (XLV).

A solution of the hydrochloride of anhydrodeoxolycodoline (600 mg) in methanol (100 ml) was shaken with Adam's catalyst (300 mg) at 45 p.s.i. hydrogen pressure for 3 hours. The mixture was filtered to remove the catalyst and the filtrate was evaporated to leave a white crystalline residue. A portion of this material was treated to recover the free base (298 mg) which was chromatographed over basic alumina (9 g). Elution with benzene (5 x 25 ml) removed the major component from the column while subsequent elution with ether (4 x 25 ml) gave a total of 40 mg of a minor component as indicated by the thin-layer chromatography (alumina; $CHCl_3$; MeOH 99:1 as eluent) used to monitor the fractions. The fraction which was the richest in the minor component (32 mg) was dissolved in acetone (5 ml) and methyl iodide (1 ml) was added. The resulting solution

was allowed to stand at room temperature for 12 hours, then excess solvent was removed and the crystalline residue was recrystallized several times from acetone to give the methiodide of deoxolycopodine (M.P. 290-291°) identical (M.P., M.M.P., superimposable infrared spectra) to an authentic sample prepared from lycopodine. (Deoxolycopodine was prepared by the Barton modification of the Wolff-Kishner reaction as described in part 3).

16. ANHYDROLYCODOLINE (XXXVIII) FROM LYCODOLINE:

A solution of lycodoline (215 mg) was dissolved in a solution of phenylphosphonic dichloride (10 ml) and pyridine (10 ml) and stirred at a solution temperature of 62-72° for 13 hours. The excess phenylphosphonic dichloride was carefully decomposed with water and the resulting aqueous solution was basified with dilute NaOH and extracted with $CHCl_3$. The dried $CHCl_3$ extract was evaporated to leave a deep red oily residue. The crude dehydration product was chromatographed over basic alumina (6 g), elution with benzene gave a colorless oily fraction (184 mg) which was homogeneous by thin-layer chromatography (alumina; benzene:$CHCl_3$, 4:1). A portion of the oily recovery was distilled (120°/0.5 mm) for a mass spectrum (M.W. = 245). The infrared spectrum (CCl_4) showed no hydroxyl absorption, relatively strong bands in the 2750 - 2850 cm^{-1} region, 1698 cm^{-1} ($\rangle C = O$) with a shoulder at 1655 cm^{-1} ($\rangle C = C\langle$). The N.M.R. spectrum ($CDCl_3$) showed a one proton triplet centered at τ = 4.44

($>C = C<^{H}$) and a 3 proton doublet at τ = 9.17 (J = 5.5 c.p.s.).

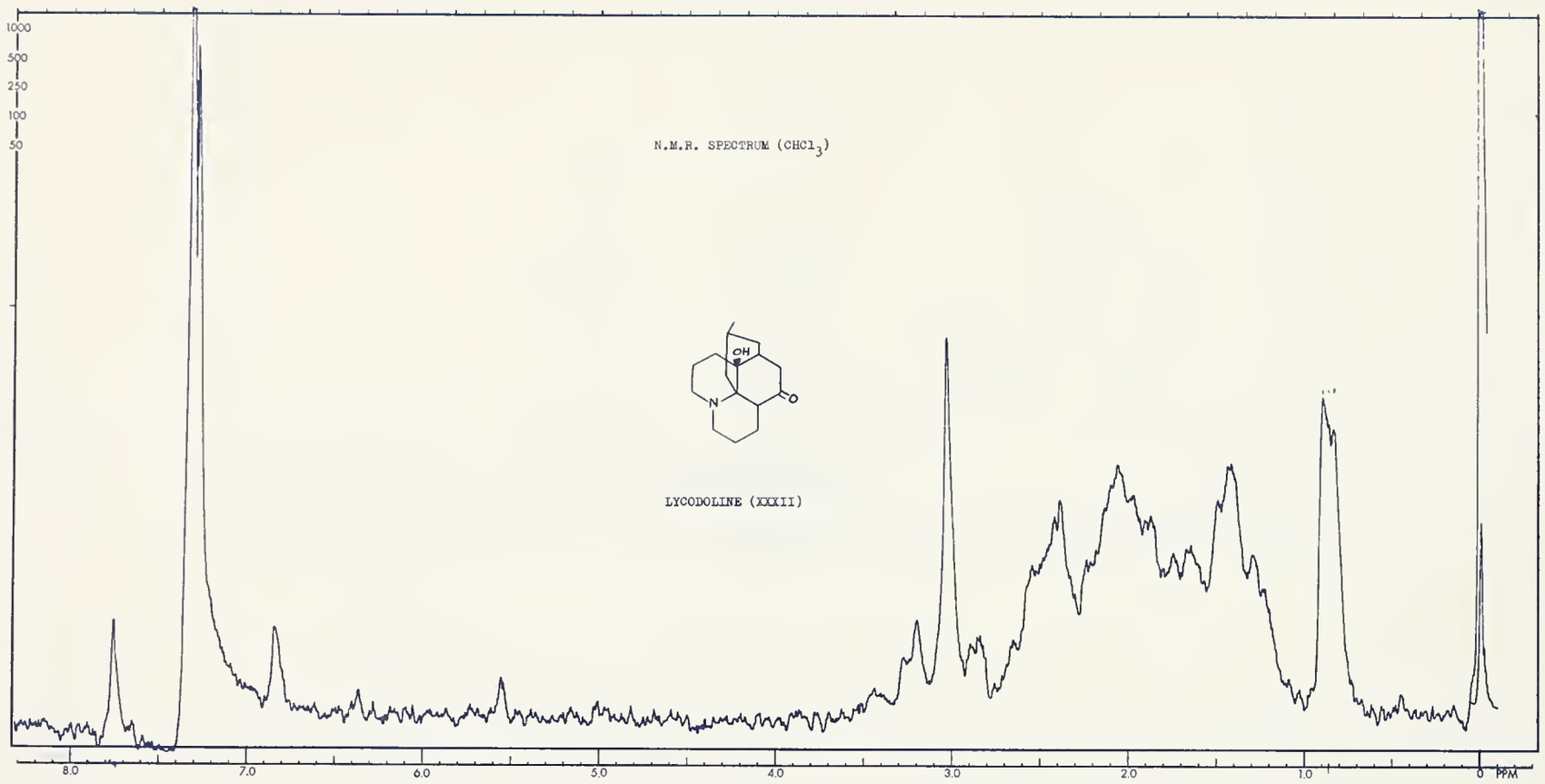
17. LYCOPODINE (XL) FROM ANHYDROLYCODOLINE (XXXVIII).

A solution of anhydrolycodoline (170 mg) in methanol was acidified with perchloric acid and shaken with Adam's catalyst (200 mg) under 45 p.s.i. hydrogen pressure for three hours. The catalyst was removed by filtration and the filtrate evaporated to a colorless crystalline residue which was dissolved in water, basified with concentrated ammonia and extracted with $CHCl_3$. The dried $CHCl_3$ extract was evaporated to yield a colorless oil which was chromatographed over basic alumina (8 g). Forty fractions (20 ml) using pentane-ether (400:1 to 9:1) as eluting solvent were taken from the column and fractions 20 to 40 were monitored by thin-layer chromatography (alumina; benzene-ethyl acetate 1:1). Fractions 30 - 32 (17 mg) which crystallized in the evaporating flasks were homogeneous (thin-layer) and were combined and recrystallized from pentane-ether to give lycopodine identical to authentic material (M.P., M.M.P., superimposable infrared spectra). The optical rotatory dispersion curve of the lycopodine prepared from lycodoline showed a positive cotton effect: $(\phi)_{305\ m\mu} = 5460^{\circ}$ $(\phi)_{258\ m\mu} = -14{,}620^{\circ}$ which compares to the optical rotatory dispersion curve of authentic lycopodine: $(\phi)_{305\ m\mu} = 6060^{\circ}$ $(\phi)_{258\ m\mu} = -14{,}200^{\circ}$.

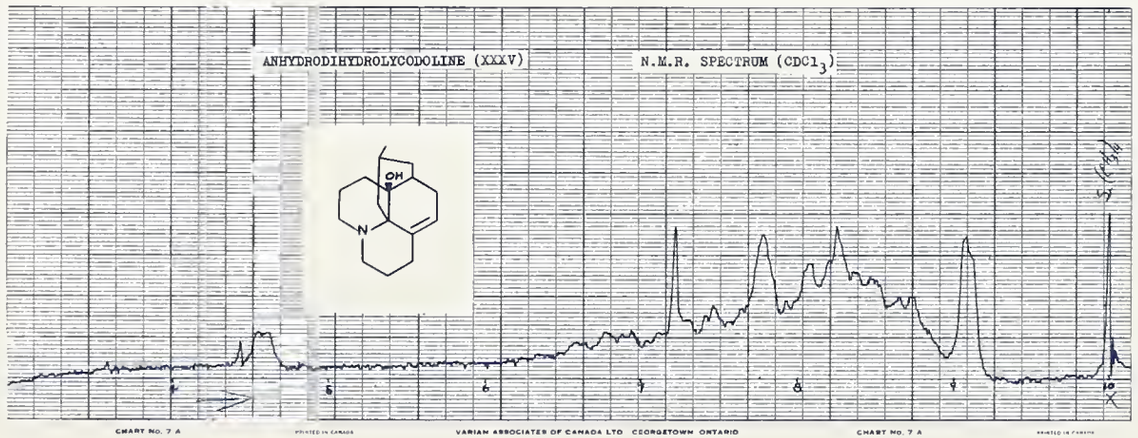
18. "EPILYCOPODINE" (XXXIX) FROM ANHYDROLYCODOLINE (XXXVIII).

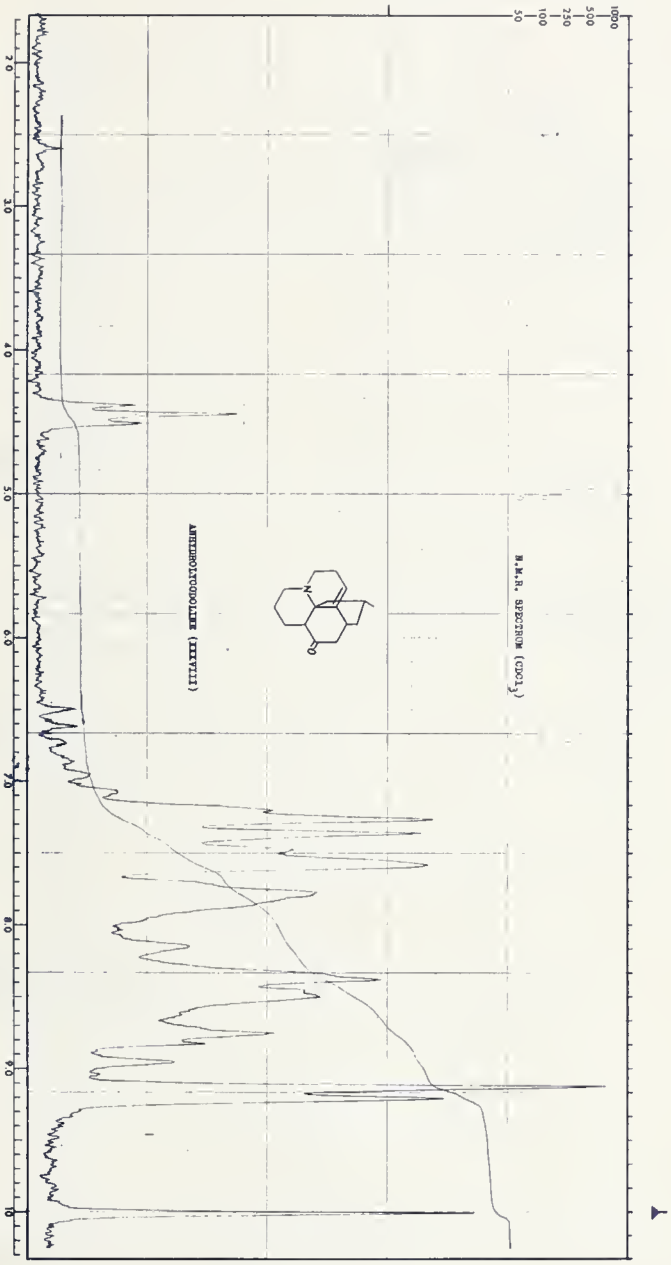
Fractions 33-39 of the chromatogram described in section 17 were combined with material from similar operations (total 153 mg) and rechromatographed over basic alumina (5 g). Elution with pentane (200 ml) removed only a trace of material from the column while elution with benzene (50 ml) gave a crystalline fraction (111 mg) which was recrystallized from pentane to constant melting point (89 - 90°). A portion was distilled (135°/0.5 mm) for analysis. (FOUND: C, 77.49, 77.78; H, 9.83, 9.99; N, 5.41. $C_{16}H_{25}ON$ requires: C, 77.67; H, 10.19; N, 5.66%). The infrared spectrum (CCl_4) showed carbonyl absorption at 1698 cm^{-1} and well developed bands in the 2750 - 2850 cm^{-1} region (page 74). The optical rotatory dispersion curve showed a negative Cotton effect

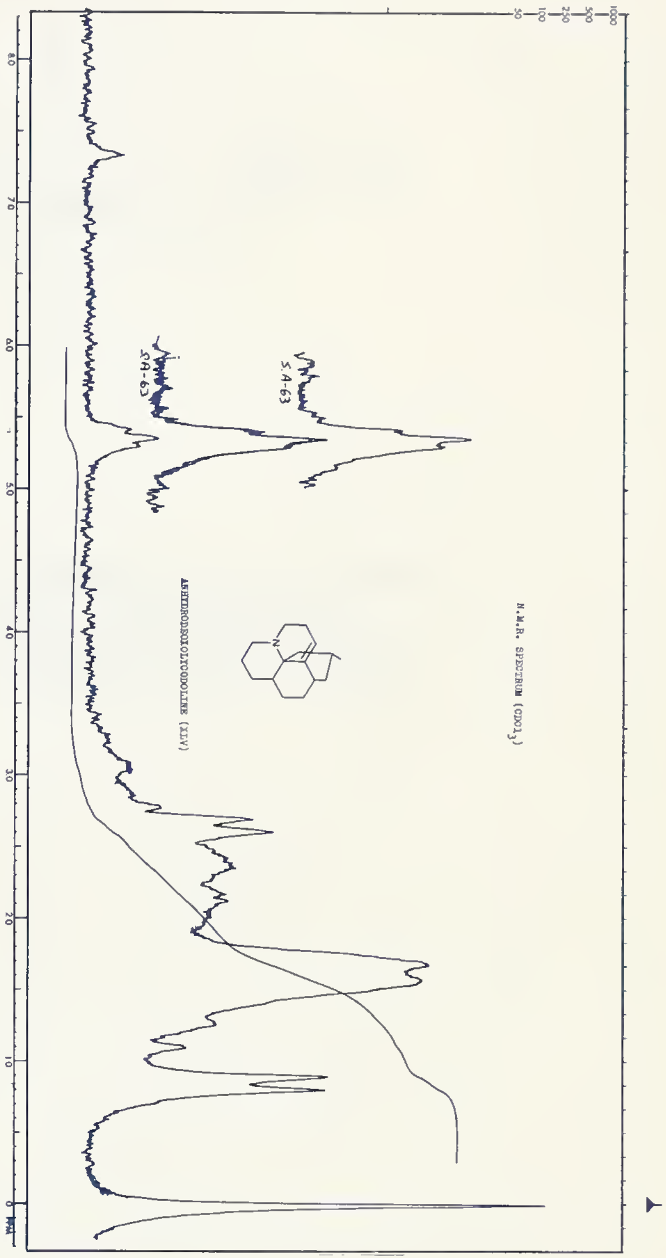
$[\phi]^{MeOH}_{306\ m\mu}$ - 2300° $[\phi]^{MeOH}_{265\ m\mu}$ + 2800°.

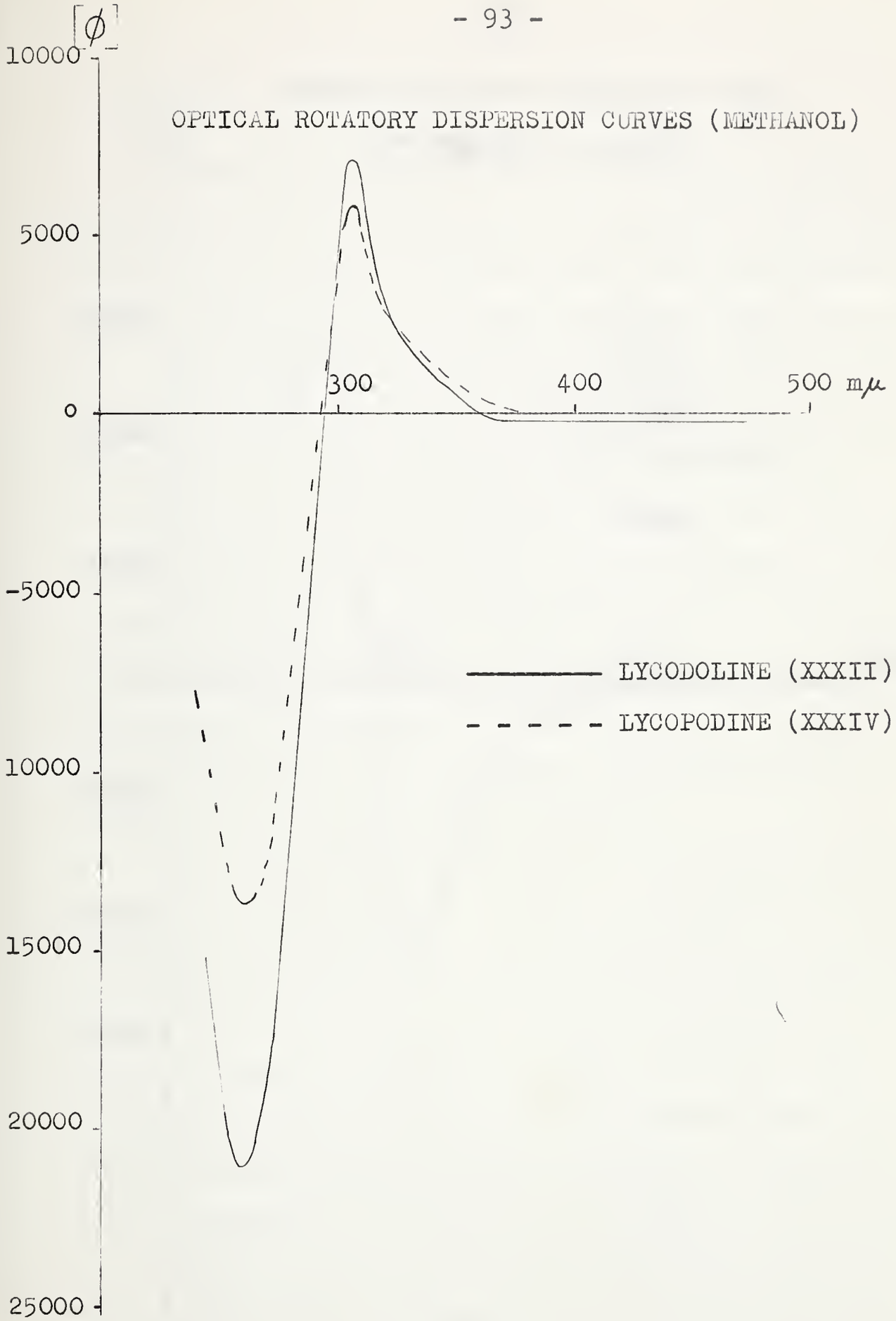


LYCODOLINE (XXXII)








[φ]
10000
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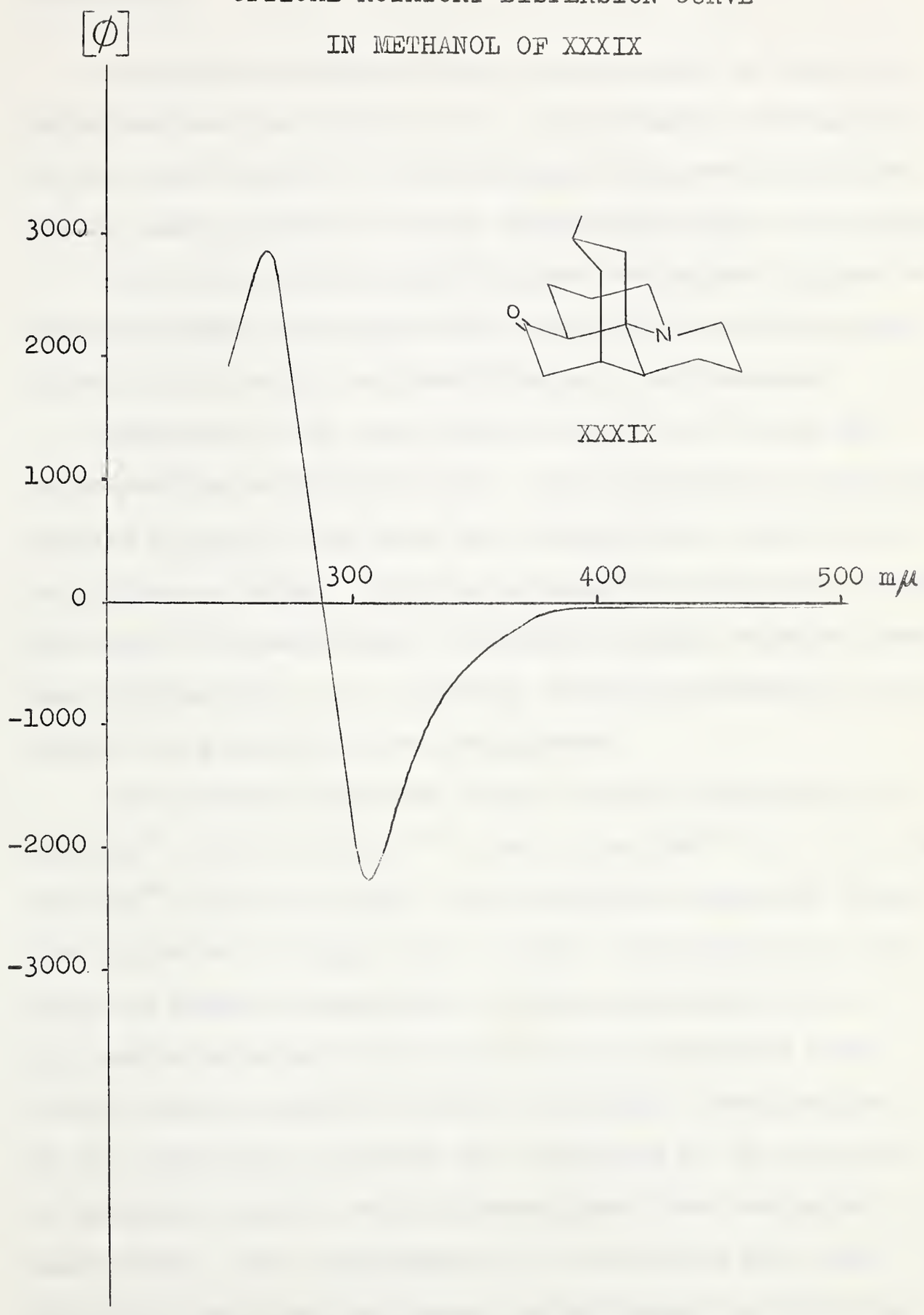
OPTICAL ROTATORY DISPERSION CURVES (METHANOL)
LYCODOLINE (XXXII)
LYCOPODINE (XXXIV)

OPTICAL ROTATORY DISPERSION CURVE

IN METHANOL OF XXXIX



XXXIX

ANNOPODINE:

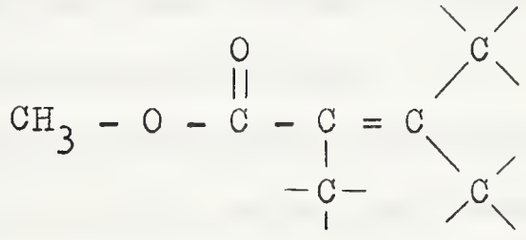
During the course of this investigation we have also characterized and carried out a preliminary investigation on the constitution of a previously unreported mononitrogenous alkaloid which we have tentatively named annopodine.

This alkaloid was isolated from the mother liquors of chromatographic fractions which had yielded α-obscurine and was purified by recrystallization from acetone.

Analyses of the pure alkaloid (M.P. 212°) and the corresponding perchlorate (M.P. 212°) suggested a molecular formula $C_{17}H_{25}O_3N$ and this was subsequently confirmed by the molecular weight (291) as indicated by the mass spectrum. Thin-layer chromatography (alumina, benzene; alumina, benzene-ethylacetate, 1:1; alumina, chloroform-methanol, 4:1) showed the alkaloid to be homogeneous.

The infrared spectrum (Nujol) showed absorption at 3300 cm^{-1} (-OH), 1710 cm^{-1} ($\rangle C=O$), 1640 cm^{-1} ($\rangle C = C\langle$), and 1225 cm^{-1} (-C-O-C-) while the ultraviolet spectrum showed absorption at 225 mμ ($\log \epsilon = 3.8$). Hydrolysis of the alkaloid (aqueous methanolic sodium hydroxide) gave an amphoteric material which could not be extracted from either aqueous acidic or basic solutions. Evaporation of the hydrolysis solution and treatment of the residue in methanol solution with diazomethane gave unchanged annopodine. This experiment, in conjunction with the spectral data presented above, establishes the presence of an α,β-unsaturated methyl ester grouping in the alkaloid.

The presence of such a group is consistent with the ultraviolet absorption spectrum (69) and the position of the carbonyl stretching vibration in the infrared spectrum. Simple esters typically absorb at somewhat higher wave number (1735 - 1750 cm^{-1}) than noted here (70). The N.M.R. spectrum shows no signals below τ = 5.9 and thus indicates that the other three positions of the olefin linkage are substituted with carbon atoms. The environment of the ester system is illustrated in partial structure I. The carbomethoxy protons appear at 6.28 τ in the N.M.R. spectrum.



I

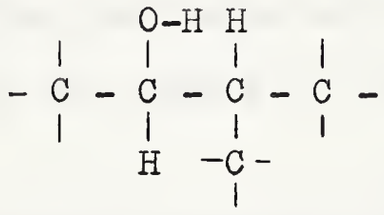
The third oxygen atom is the form of a hydroxyl group as indicated by the infrared spectrum (nujol, 3300 cm^{-1}) and confirmed by the solution spectrum (CCl_4) which shows the typical free hydroxyl stretching vibration at 3625 cm^{-1}. Treatment of annopodine with pyridine-acetic anhydride gave a basic o-acetyl derivative which shows a one proton signal at τ = 4.84 in the N.M.R. spectrum, apparently replacing a one proton signal at τ = 5.94 in the N.M.R. spectrum of annopodine. This data is consistent with the presence in annopodine of an easily acetylated secondary hydroxyl group (26). This

is supported to some extent by the results obtained from the oxidation of annopodine with chromic acid-acetic acid. The infrared spectra ($CHCl_3$, CCl_4) of fractions obtained by chromatography of the crude reaction product were free of hydroxyl absorption but showed carbonyl bands at 1710 and 1698 cm^{-1}. The ultraviolet absorption spectrum of a representative fraction showed a well defined band at 228 mμ. The spectral data is consistent with the retention of the α,β-unsaturated ester system and the oxidation of a secondary hydroxyl group to a ketone which forms part of a six (or larger) membered ring. Since the product was not further characterized the evidence gained from the reaction is suggestive but not definitive.

The acetylation experiment shows that the nitrogen atom in annopodine is tertiary since the O-acetyl derivative is basic and the infrared spectrum shows no absorption in the N-H stretching region. The molecular formula and the nature of the functional groups require the molecule to be tetracyclic.

It was found that annopodine could be dehydrated efficiently by $POCl_3$-pyridine. The infrared spectra (CCl_4) of the fractions obtained by elution chromatography of the crude product were free of hydroxyl absorption but showed carbonyl absorption (1710 cm^{-1}) and olefinic absorption (1640 cm^{-1}). The unstable oil analyzed reasonably well for the expected anhydro compound ($C_{17}H_{23}O_2N$) and the N.M.R. spectrum showed a signal at τ = 4.52 (unresolved multiplet). The signal at

τ = 4.52 cannot represent more than one hydrogen atom since it is one quarter to one third as intense as the sharp signal at τ = 6.30 which must represent the methyl protons of the carbomethoxy group. It follows that the dehydration takes place towards an α carbon which carries a single hydrogen. Thus part structure II must be incorporated in annopodine.



II

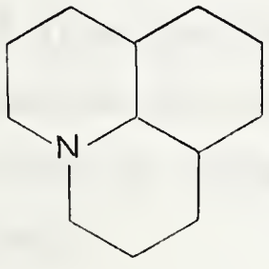
The anhydro compound does not exhibit strong absorption above 225 mμ in the ultraviolet spectrum which shows that the olefin produced by the dehydration is not in conjugation with the α,β- unsaturated ester. This means that the carbon bearing the hydroxyl group in annopodine is either isolated (in terms of intervening carbon atoms) from the α,β- unsaturated system, or that some structural feature such as a bridgehead or an inadequate stereochemical situation prevents the formation of a conjugated system. The hydroxyl cannot be allylic to the double bond.

The N.M.R. spectrum of annopodine shows, besides the three proton signal at τ = 6.28 (singlet, O-CH_3), a three proton signal at τ = 8.96 (doublet, J = 5 c.p.s.) indicative of a -$CHCH_3$ group which is supported by the production of less than one mole of volatile acid on Kuhn-Roth oxidation.

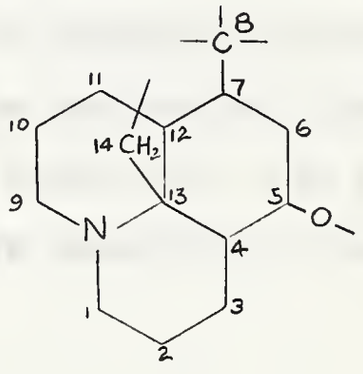
Since the anhydro compound also shows a doublet in the N.M.R. (τ = 8.84; J = 6 c.p.s.) the hydroxyl group cannot be α to the methyl group.

Since the available annopodine was largely consumed in this preliminary investigation, it has not been possible to extend our knowledge of the alkaloid beyond what has been presented. However annopodine is a tetracyclic compound incorporating a tertiary nitrogen and a secondary C-methyl group; features which are common to almost all Lycopodium alkaloids.

The mononitrogeneous Lycopodium alkaloids of known structure all possess a hexahydrojulolidine ring system (III), invariably substituted as shown in Figure IV.

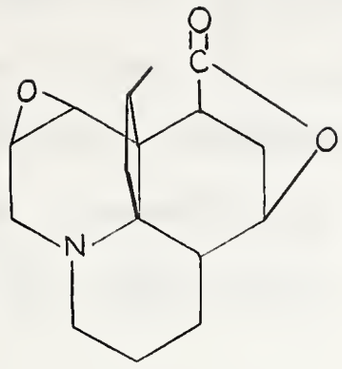


III

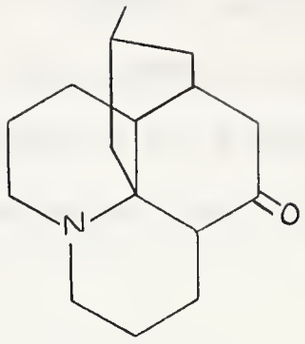


IV

The features of Figure IV are incorporated into two structural types; one of which is solely represented by annotinine (V), and the other by lycopodine VI.



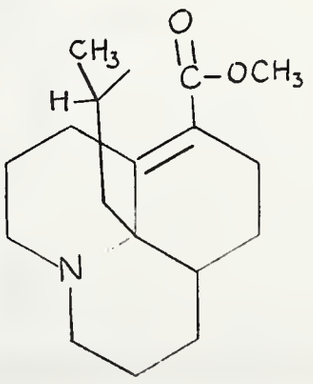
V



VI

The other mononitrogeneous Lycopodium alkaloids of known structure are peripheral variants of lycopodine and include various oxidation states at C-4, C-5, C-6, C-8, and C-11-12 with oxidation at C-8 being most frequently observed.

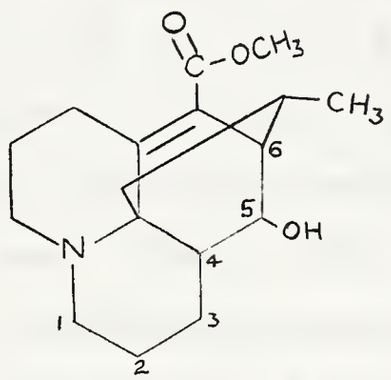
If the assumption is made that annopodine does not depart from the hexahydrojulolidine pattern and does indeed incorporate the features shown in Figure IV; then part structure VII becomes an attractive possibility for the alkaloid.



+ -OH AND ONE MORE RING

VII

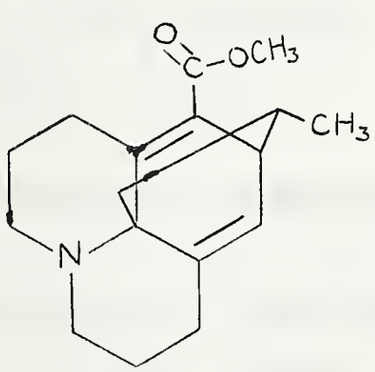
Since the Lycopodium alkaloids are always oxygenated at C-5 and it has been shown above that the hydroxyl group in annopodine is attached to a carbon atom ∝ - to a methine carbon, a possibility for annopodine becomes the structure shown in igure VIII.



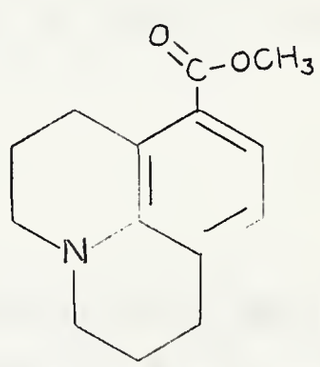
VIII

The bridge ring is attached to C-6 to prevent conjugation in anhydroannopodine.

If structure VIII is correct for annopodine then anhydroannopodine would be represented by Figure IX and



IX



X

when further supplies of the alkaloid become available, the structure proposal may be quite easily tested since IX should undergo a facile reverse Diels-Alder reaction (thermally or in the mass spectrometer) to give X.

EXPERIMENTAL ANNOPODINE.

1. Annopodine was initially isolated from the mother liquors of fractions which had yielded α -obscurine. Recrystallization from acetone gave the analytical sample M.P. 211 - 212°. (FOUND: C, 69.82, 69.57, 69.75; H, 8.73, 8.73, 8.67; O, 16.62; N, 4.68; $-CH_3$, 3.23; $C_{17}H_{25}O_3N$ requires: C, 70.06; H, 8.65; O, 16.47; N, 4.81; $-CH_3$, 5.15%). Infrared spectrum (nujol) 3300 cm^{-1} (OH), 1710 cm^{-1} ($\rangle C=O$), 1640 cm^{-1} ($\rangle C=C\langle$), 1225 cm^{-1} (-C-O-C-). Ultraviolet spectrum λ max 225 mμ log ϵ = 3.8. N.M.R. spectrum τ = 8.96 (centre of doublet, J = 5.c.p.s., 3-proton), τ = 6.28 (singlet, 3-proton).

Formation of the perchlorate in acetone solution followed by recrystallization from acetone gave the analytical sample M.P. 210 - 212°. (FOUND: C, 52.18, 52.32; H, 6.74, 6.81; O, 28.17; N, 3.50; Cl, 8.89; $C_{17}H_{25}O_3N.HClO_4$ requires: C, 52.10; H, 6.19; O, 28.58; N, 3.57; Cl, 9.05%).

2. HYDROLYSIS OF ANNOPODINE:

A solution of annopodine (88 mg) in 0.5N NaOH (aqueous methanol 3:2) was refluxed for one hour and allowed to stand at room temperature for 18 hours. Chloroform extraction of the hydrolysis solution followed by continuous ether extraction of acidified hydrolysis solution demonstrated the amphoteric nature of the hydrolysis product.

The amorphous residue remaining after evaporation of the acidified hydrolysis product was dissolved in methanol and treated with ethereal diazomethane. The excess solvents were removed and the oily residue was dissolved in dilute HCl. The aqueous acid solution was washed with ether, basified with concentrated ammonia, and extracted with $CHCl_3$. The dried $CHCl_3$ extract was evaporated to leave an oily residue (54 mg) which was crystallized from acetone solution to give annopodine identical to starting material.

3. THE ACETYLATION OF ANNOPODINE:

A solution of annopodine (100 mg), pyridine (15 ml), and acetic anhydride (5 ml) was kept at room temperature for 24 hours. The reaction solution was evaporated to leave an oily residue from which the basic component (88 mg) was isolated by acid-base extraction. Formation of the perchlorate in acetone solution and recrystallization from acetone-ethyl acetate gave the analytical sample M.P. 250 - 252°. (FOUND: C, 52.73, 53.03; H, 6.71, 6.42; O, 28.99; $C_{19}H_{27}O_4N.HClO_4$ requires: C, 52.71; H, 6.29; O, 29.56%). Infrared spectrum (nujol), 1735 cm^{-1} (acetate), 1695 cm^{-1} (ester), 1640 cm^{-1} (>C=C<). The free base recovered from the purified perchlorate showed ultraviolet absorption λ max 222 mμ log ϵ = 4.04. The N.M.R. spectrum showed absorption at τ = 8.91 (doublet, J = 5.5 c.p.s. - 3 protons - $CHCH_3$), τ = 7.97 (singlet, 3-protons, acetate), τ = 6.28 (singlet, 3-protons, $-OCH_3$), and

τ = 4.84 (1-proton H-C-OAc).

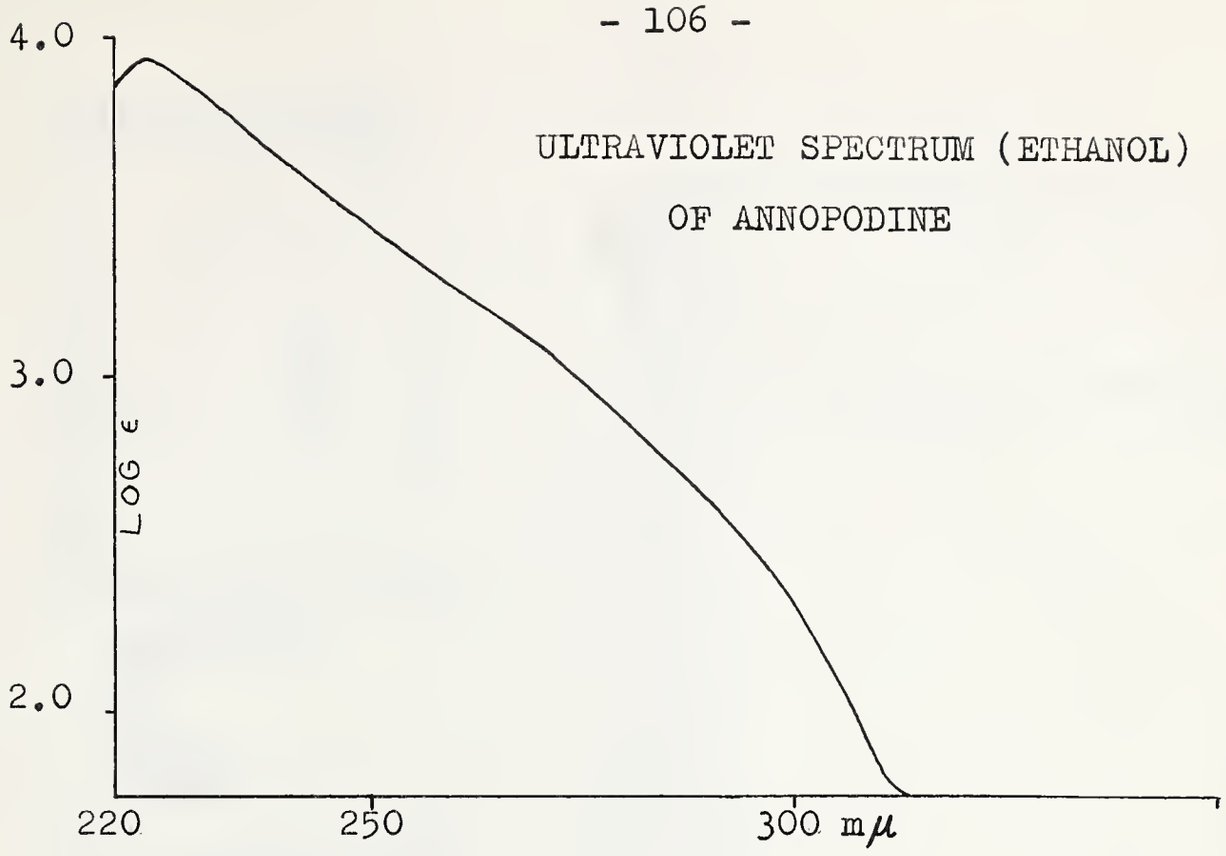
4. THE DEHYDRATION OF ANNOPODINE:

Annopodine (50 mg) in pyridine (5 ml) and $POCl_3$ (1 ml) was allowed to stand at room temperature for 24 hours. The reaction solution was evaporated to leave an oily residue from which the basic material (48 mg) was isolated by acid-base extraction. Chromatography over neutral alumina (activity I, 2 g) and elution with benzene gave an oily fraction which was free of hydroxyl absorption in the infrared spectrum but showed absorption at 1710 cm^{-1} (>C=O), 1640 cm^{-1} (>C=C<) and 1240 cm^{-1} (-C-O-C-). A portion was distilled (110° - 0.5 mm) for analysis. (FOUND: C, 74.41, 74.36; H, 9.01, 8.64; O, 11.59; $C_{17}H_{23}O_2N$ requires: C, 74.69; H, 8.48; O, 11.70%). The N.M.R. spectrum (of further anhydroannopodine prepared as described above) showed absorption at τ = 8.84 (doublet, J = 6 c.p.s., 3-protons), τ = 6.30 (singlet, 3-protons), τ = 4.52 (1-proton) assigned to $-CHCH_3$, $-O-CH_3$, and >C=C<H respectively.

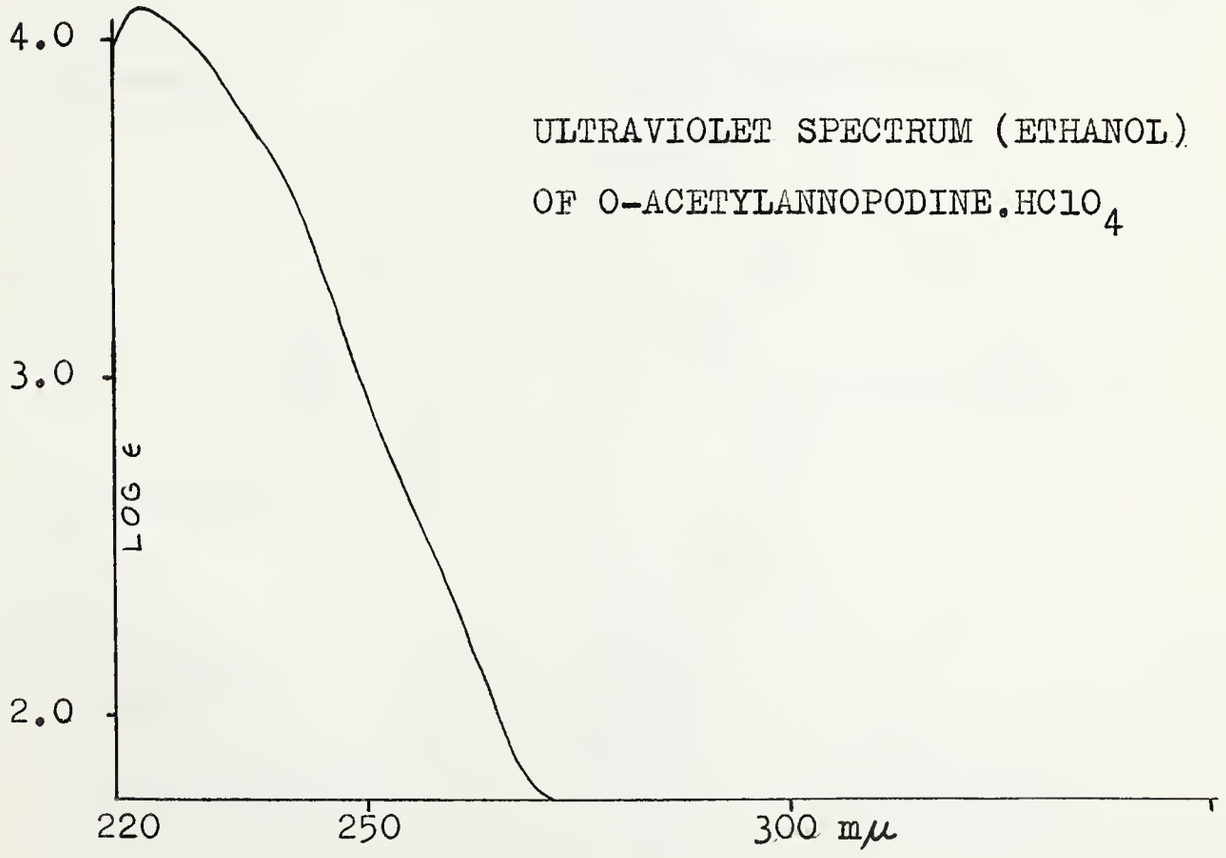
5. OXIDATION OF ANNOPODINE:

Annopodine (35 mg) in HOAc (1.5 ml) was added to a solution of CrO_3 (40 mg) in HOAc (1.5 ml) and H_2O (0.2 ml). The reaction mixture was stirred at room temperature for 4½ hours, diluted with methanol, and evaporated to leave an amorphous residue which was subjected to acid-base purification to yield an oily residue (23 mg). Chromatography

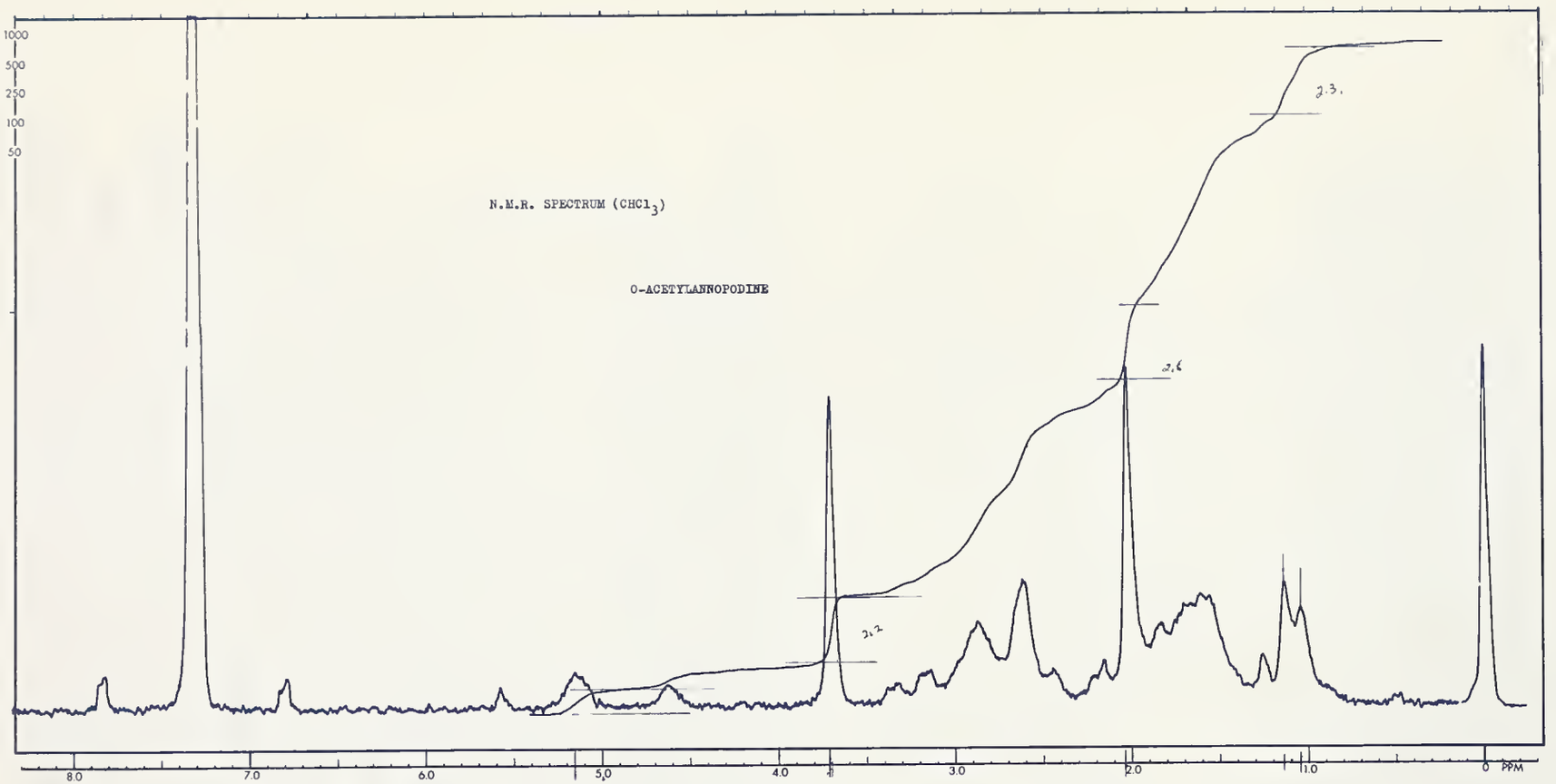
over basic alumina (0.5 g) and elution with ether, CH_2Cl_2 and $CHCl_3$ (20 ml) gave similar fractions (5 mg each) which were free of -OH absorption in the infrared spectra and showed carbonyl absorption at 1710 and 1698 cm^{-1}. The ultraviolet absorption spectrum of the combined fractions showed a maximum at 228 mμ. This reaction was not repeated nor were attempts made to further characterize the product.

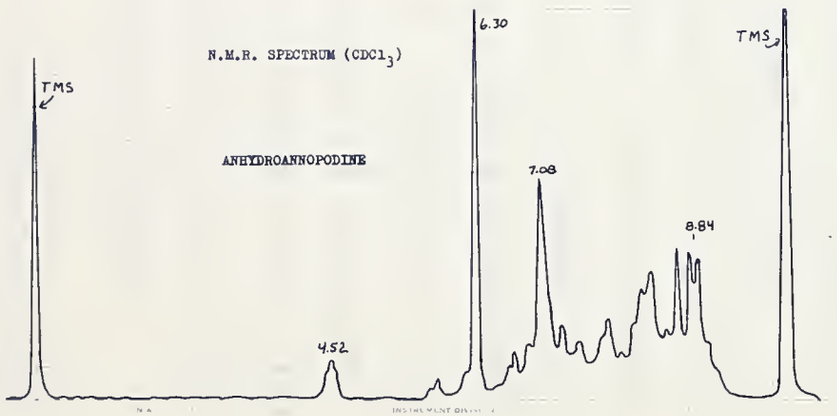


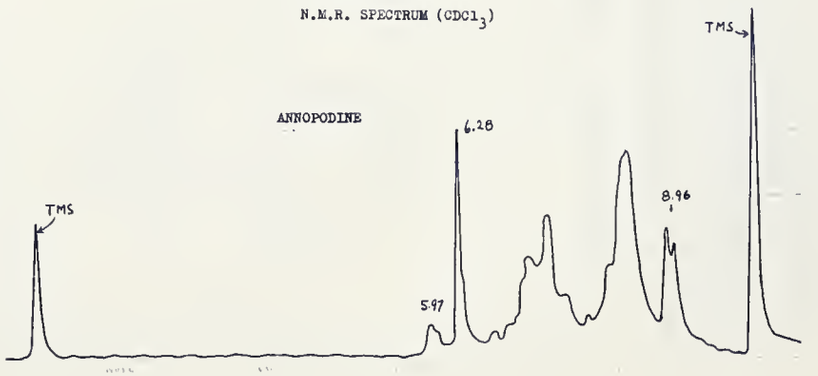
ULTRAVIOLET SPECTRUM (ETHANOL)
OF ANNOPODINE



ULTRAVIOLET SPECTRUM (ETHANOL)
OF O-ACETYLANNOPODINE.$HClO_4$


1000
500
250
100
50
N.M.R. SPECTRUM ($CHCl_3$)
O-ACETYLANNOPODINE
2.3
2.6
2.2
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0 PPM

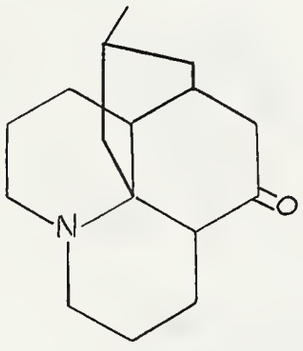

N.M.R. SPECTRUM ($CDCl_3$)
ANHYDROANNOPODINE
TMS
TMS
6.30
7.08
8.84
4.52


N.M.R. SPECTRUM ($CDCl_3$)
ANNOPODINE
TMS
TMS
6.28
8.96
5.97

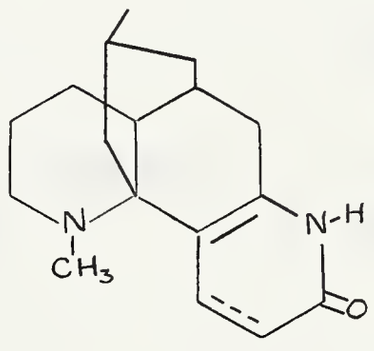
THE BIOSYNTHESIS OF THE LYCOPODIUM ALKALOIDS:

Since we have made formal use of biosynthetic concepts as an aid in arriving at the correct structures for some of the alkaloids discussed in preceding sections, it seems pertinent to discuss this aspect of Lycopodium chemistry in some detail. It should be pointed out that we have not carried out any experimental work directed towards the elucidation of the biosynthesis of these alkaloids and therefore the following arguments properly belong in the realm of conjecture.

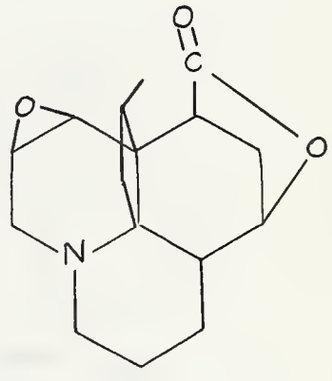
The Lycopodium alkaloids of known structure can be divided into three groups, based on the structures of lycopodine (I), the obscurines (II), and annotinine (III).



I

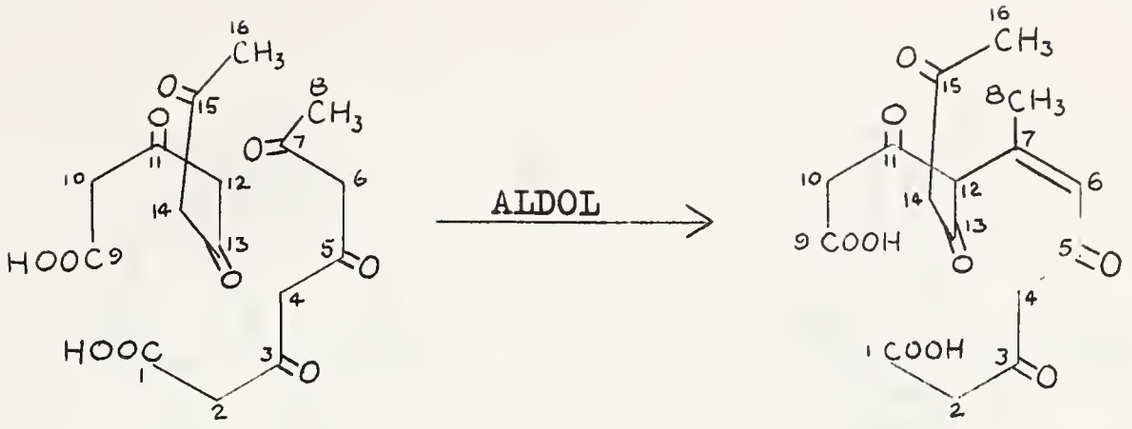


II



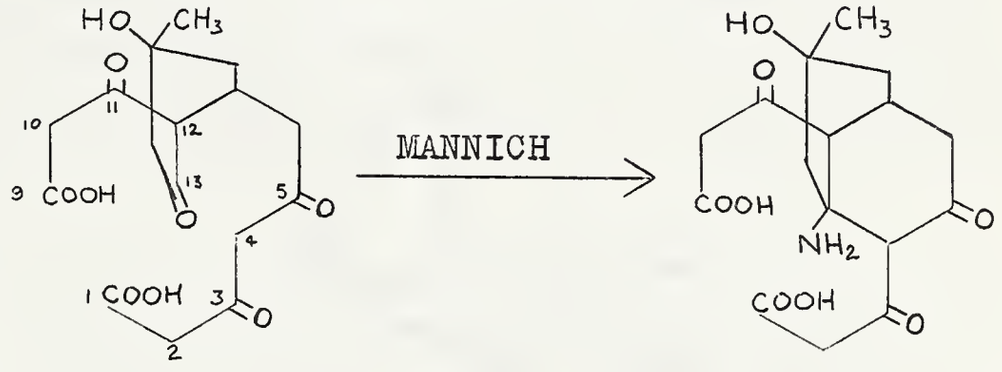
III

In 1960, Conroy (74) proposed that these alkaloids could be formed in the plant by the condensation of two 3,5,7-triketo-octanoic acid chains (IV) which, in turn, are derived from acetate units.



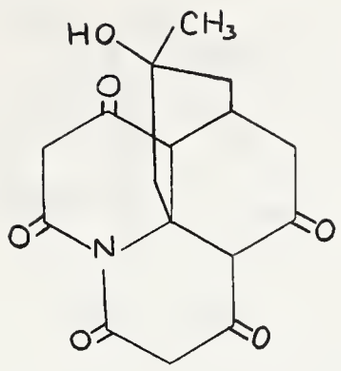
IV V

An initial aldol condensation between the methylene at C-12 and the C-7 carbonyl (Figure IV) followed by dehydration leads to V which may be further elaborated to VI by a second aldol condensation between C-8 and the C-15 carbonyl.

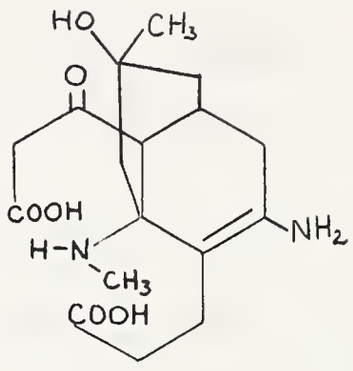


VI VII

A Mannich reaction on VI involving the C-13 carbonyl, the C-4 methylene and ammonia leads to the nitrogeneous intermediate VII which upon lactam formation gives the immediate precursor VIII of the lycopodine-type alkaloids.

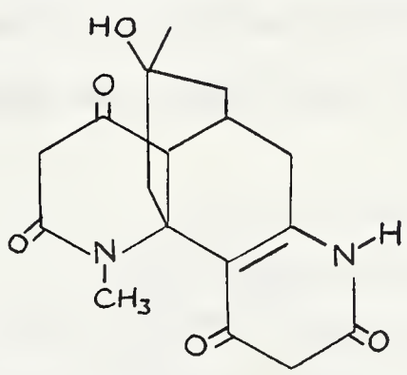


VIII



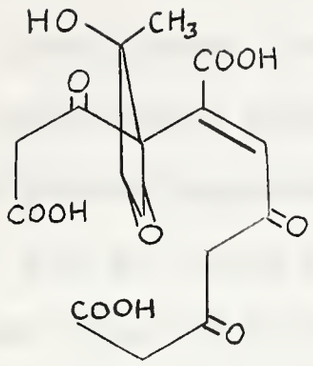
IX

Conroy's scheme provides the obscurine-type alkaloids by modification of VII to an intermediate such as IX (condensation of the C-5 carbonyl with ammonia, dehydration, and N-methylation) followed by appropriate lactam closure to the immediate precursor X of the obscurines.

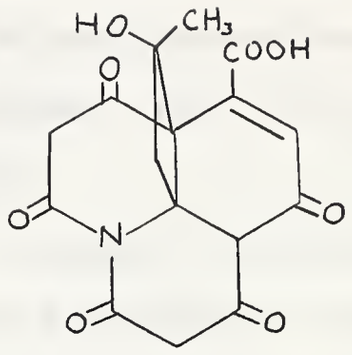


X

Oxidation of the C-8 methyl group to carboxyl in Figure V followed by closure between C-12 and C-15 leads to an intermediate XI containing the cyclobutane ring. Mannich condensation (ammonia, C-13 carbonyl and C-4 methylene) followed by lactam formation in the same way.



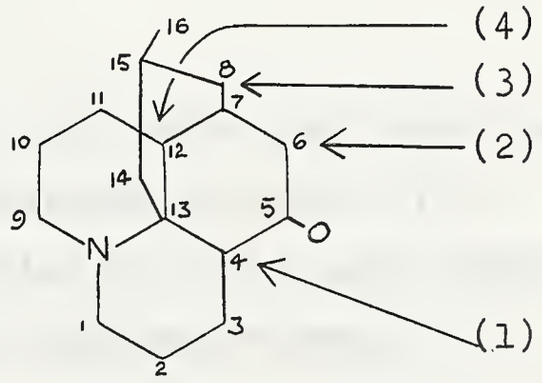
XI



XII

as that described for the lycopodine-type alkaloids leads to a carbon skeleton of the annotinine type.

Although the gross features of the Lycopodium alkaloids are convincingly provided by the acetate scheme, it was proposed when lycopodine was the sole representative of that structural type. Since that time a number of structures have been developed which incorporate the entire lycopodine molecule but display one additional site of oxygenation.



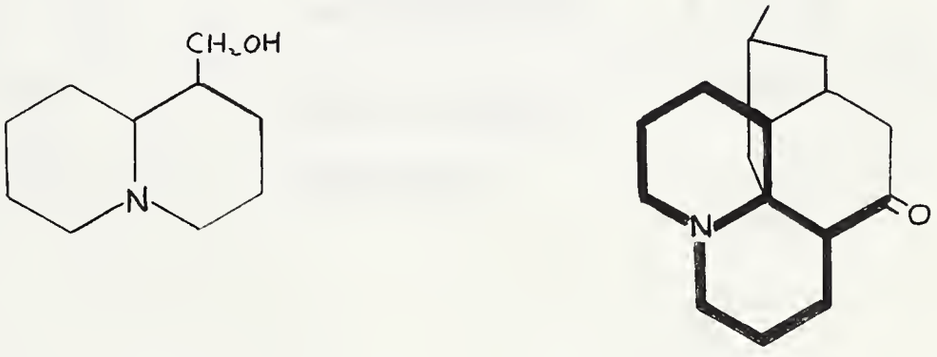
XIII

The keyed arrows of figure XIII indicate these additional sites of oxygenation and refer to the following specific

examples: (1) flabelliformine (4-hydroxylycopodine) (71); (2) lycoclavine (6-hydroxy-O-acetyldihydrolycopodine (14); (3) annofoline (8-ketodihydrolycopodine) (72); (4) lycodoline (12-hydroxylycopodine) (this thesis).

All of these structures may of course be derived from figure VIII by a series of selective reductions, dehydrations, hydrations and oxidations but we felt that some other scheme might explain the observed oxidation pattern more convincingly.

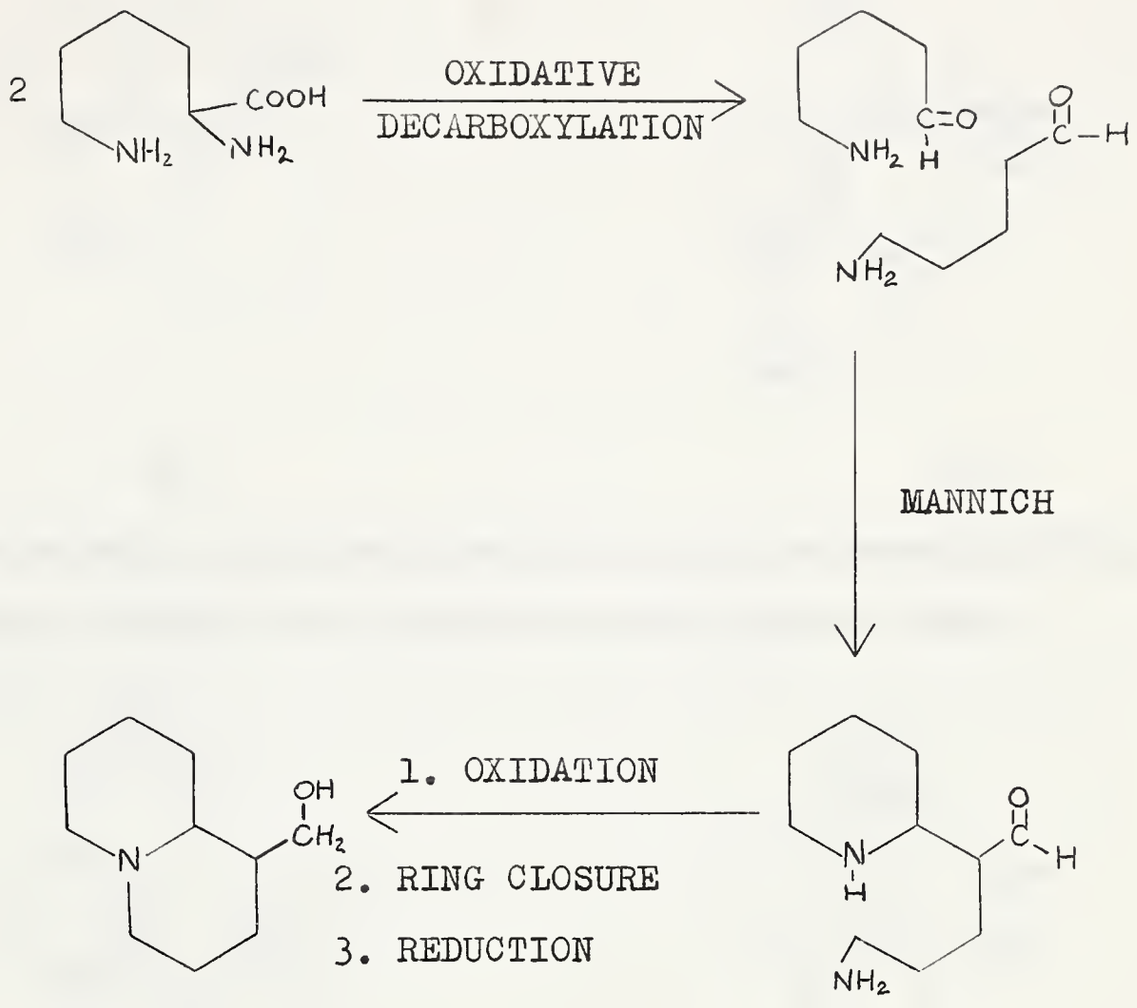
It has been pointed out (40a) that the carbon skeleton of lupinine (XIV) is included intact in the carbon skeleton



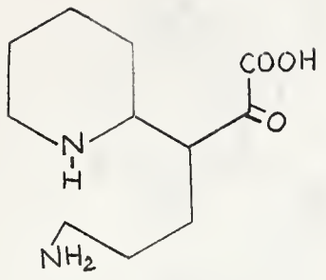
XIV

of lycopodine and it is known that lysine is a biogenetic precursor of the Lupinine alkaloids (73).

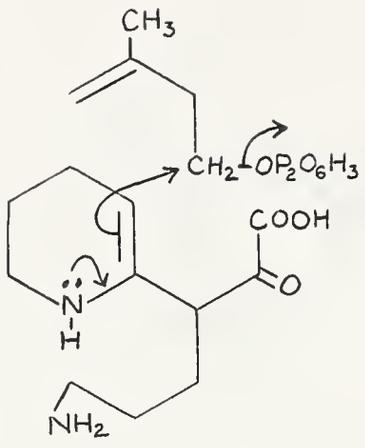
The incorporation of lysine into lupinine may be represented by the following scheme:



The scheme for lupinine could lead to an intermediate XV by the condensation of one molecule of amino aldehyde with a modification of lysine obtained by a transamination reaction rather than an oxidative decarboxylation. Oxidation of XV to the enamine XVI followed by alkylation with

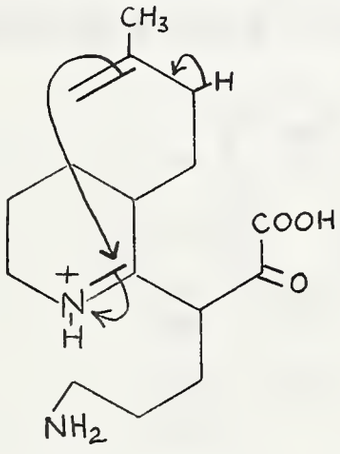


XV

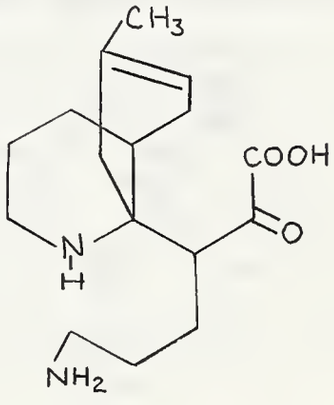


XVI

isopentenyl pyrophosphate leads to an intermediate XVII which contains the required sixteen carbon atoms.



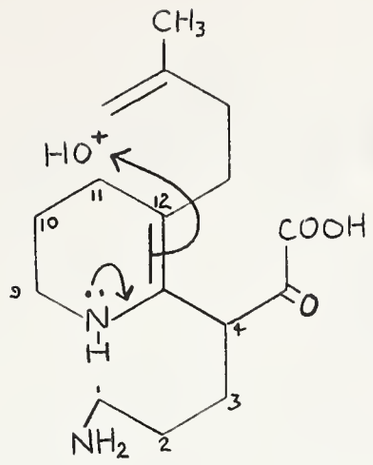
XVII



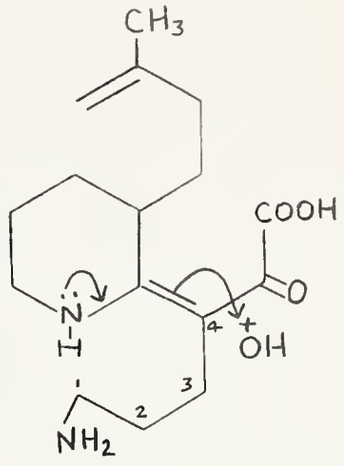
XVIII

Nucleophilic attack by the double bond on the protonated C - N double bond gives the bicyclic intermediate XVIII.

Two enamines XIX and XX corresponding to XVII can be written which, upon appropriate oxidation, easily rationalize the appearance of hydroxyl at C-4 and C-12 in the lycopodine skeleton.

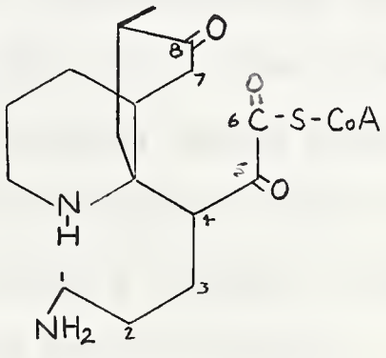


XIX

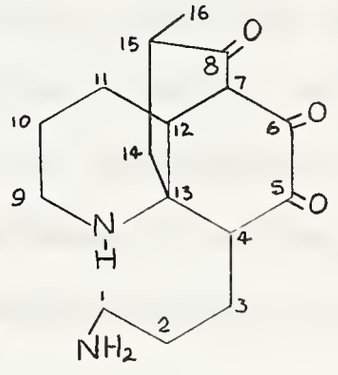


XX

Elaboration of the double bond in the bicyclic intermediate XVIII to a ketone and activation of the carbonyl group as in XXI followed by a Claisen type condensation



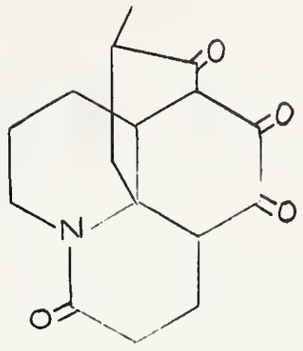
XXI



XXII

(involving C-6 and C-7) could lead to the tricyclic intermediate XXII.

Oxidation of the carbon atom carrying the primary amino group and subsequent lactam closure provides XXIII as an immediate precursor of the lycopodine type alkaloids.

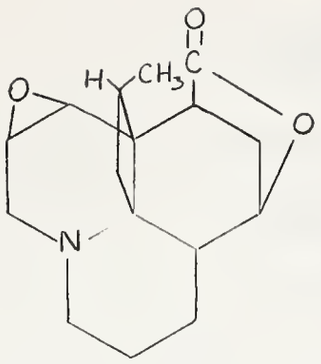


XXIII

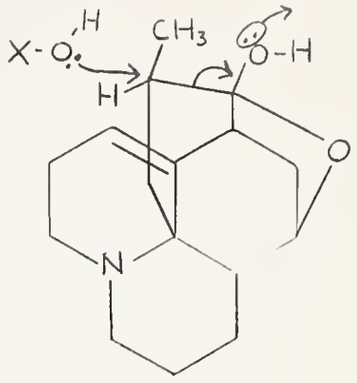
Since the scheme could be carried through with a hydroxyl group at C-4 or C-12, the predicted oxygenation pattern compares favorably with that observed (Figure XIII).

The obscurine-type alkaloids could arise from intermediate XXII by a ring closure involving the C-5 carbonyl and the primary amino group followed by oxidation at C-1 or by elaboration of XXII to an intermediate analogous to IX in Conroy's scheme followed by lactam closure and reduction at C-6 and C-8.

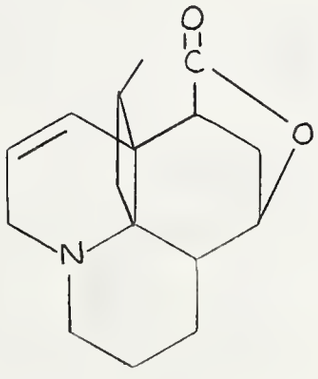
Since annotinine appears to be the sole representative of its class, it may be argued that it must be formed at a later, rather than an earlier, stage of the biosynthesis. The similarity between annotinine (III) and acrifoline in the hemi-ketal form (XXIV) has been pointed out (40a) and in view of this, a possible mode of formation is outlined by structures XXIV to XXVI.



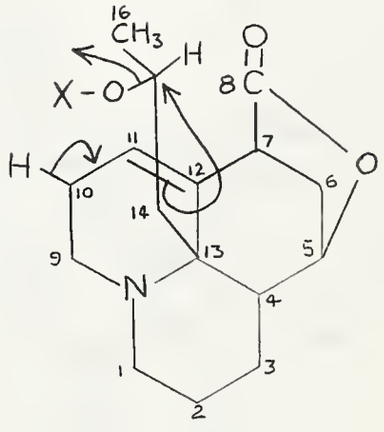
III



XXIV

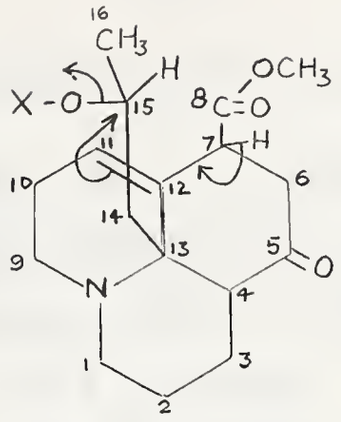


XXVI

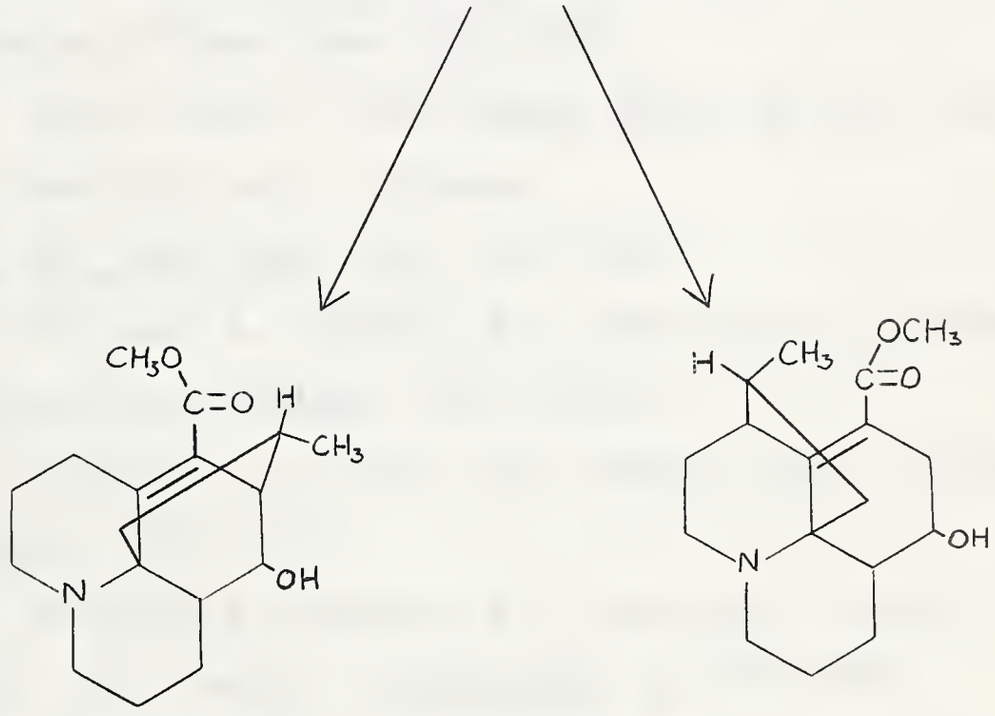


XXV

Elaboration of XXV by lactone opening, esterification, and oxidation at C-5 leads to XXVII. A Claisen type reaction between C-6 and C-15 followed by isomerization of the C11-12 double bond into conjugation with the ester and C-5 reduction leads to the tentative structure proposed earlier for annopodine. Alternatively, loss of the C-7 hydrogen and



XXVII



XXVIII

C 11 - 15 bond formation (as shown in XXVII) leads to XXVIII, an equally plausible structure for annopodine.

B I B L I O G R A P H Y

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